Transcriptomic Analyses Reveal the Effect of Nitric Oxide on the Lateral Root Development and Growth of Mangrove Plant *Kandelia Obovata*

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Research Article

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Abstract

Background and aims *Kandelia obovata*, a dominant mangrove species in China, produces complex buttress roots and prop roots in intertidal wetlands where high quantities of nitric oxide (NO) are produced by reducing sediments. NO, a key signaling molecule, participates in an array of plant physiological and developmental processes. However, it is unclear whether NO functions in *K. obovata* root system establishment.

Methods Here, we used a transcriptomic approach to investigate the potential role of NO in the regulation of *K. obovata* lateral root development and growth. Transcript profiles and bioinformatics analyses were used to characterize potential regulatory mechanisms.

Results NO enhanced *K. obovata* lateral root development and growth in a dose-dependent manner. RNA-seq analysis identified 1,593 differentially expressed genes (DEGs), of which 646 and 947 were up- and down-regulated in roots treated with NO donor. Functional annotation analysis demonstrated that the starch and sucrose pathway was significantly induced in response to NO. A suite of DEGs involved in hormone signal transduction and cell wall metabolism was also differentially regulated by NO. Taken together, our results suggest that a complex interaction between energy metabolism, multiple hormone signaling pathways, and cell wall biosynthesis is required for the NO regulation on lateral root development and growth in mangrove plant *K. obovata*.

Conclusion NO appears to contribute to the formation of the unique root system of mangrove plants.

Introduction

Nitric oxide (NO) is a small gaseous signaling molecule that has aroused the interest of plant scientists during the last decade because of its versatile roles in multiple biotic and abiotic stresses, including heavy metal stress (Li et al. 2018), waterlogging (Chen et al. 2016), drought (Mata and Lamattina 2001), and high salinity (Shen et al. 2018). NO also participates in various plant physiological and developmental processes, such as floral transition (He et al. 2004), stomatal movement (Wilson et al. 2008), and seed germination (Libourel et al. 2006).

Increasing evidence suggests that NO participates in plant root development and growth. For example, low concentrations of sodium nitroprusside (SNP), an NO donor, induce root growth and increase lignification in soybean seedlings (Böhm et al. 2010) and inhibit root elongation in maize (Zhao et al. 2007). NO was reported to promote waterlogging tolerance in the euhalophyte *Suaeda salsa* by enhancing adventitious root formation through increases in endogenous NO production and nitric oxide synthase (NOS) activity (Chen et al. 2016). Moreover, NO mediates downstream signaling during H₂-induced adventitious root organogenesis in cucumber (Zhu et al. 2016) and is the key signal in cell wall remodeling of *Arabidopsis* root hair cells (Moro et al. 2017). In addition, NO helps to regulate lateral root development in tomato, probably operating within the auxin signal transduction pathway (Correa-Aragunde et al. 2004). NO is required for indole-3-butyric acid (IBA)-triggered adventitious rooting in

Phaseolus radiatus L. (Huang et al. 2007). Sun et al. (2016) reported that NO-activated seminal root elongation in rice under nitrogen and phosphate deficiencies is associated with another group of plant hormones, strigolactones. Extensive physiological and biochemical studies on the role of NO in root development have been conducted in recent decades. However, the transcriptional changes and molecular mechanisms by which NO mediates lateral root establishment require further exploration.

Mangrove forests are the dominant intertidal ecosystems along tropical and subtropical coastlines around the world (Lin 1999). They play irreplaceable ecological roles in the overall coastal ecosystem, providing critical ecosystem services such as coastal protection, biodiversity support, habitat provision, and water quality improvement (Lin 1999). To survive in habitats with chronic periodic flooding, mangrove plants have developed complex root systems with specialized characteristics, including various forms of aerial roots with pneumatophores in *Avicennia marina*, plank roots in *Xylocarpus grantum*, and stilt roots in *Rhizophora mucronate* (Naidoo et al. 1997, Youssef and Saenger 1996).

Kandelia obovata, the “true mangrove”, belongs to the family Rhizophoraceae and is regarded as a viviparous plant (Chen et al. 1995). The normal sexual reproduction of *K. obovata* produces non-dormant embryos that grow primarily by hypocotyl elongation to form cigar-shaped seedlings, which remain attached to the parent trees for several months (Chen et al. 1995). After the hypocotyls drop off into the mud, a root system gradually develops with both complex buttress roots and prop roots.

Another important characteristic of the mangrove wetland is the high quantity of NO produced in the reducing sediments (Chen et al. 2010). Nonetheless, it is unclear whether this NO functions in *K. obovata* root system establishment. Likewise, few genomic resources are available to study patterns of gene expression and molecular mechanisms that underlie this process. In this study, we used a transcriptomic approach to investigate the possible function of NO in the modulation of *K. obovata* lateral root development and growth.

De novo transcriptome sequencing based on high-throughput technology has been successfully employed to analyze gene expression in non-model plant species and serves as a powerful tool for revealing the molecular basis of multiple biological processes (Li et al. 2018, Wei et al. 2014). This technology has been used in previous studies of root formation. For example, Wei et al. (2014) demonstrated that genes associated with plant hormone signal transduction, cell wall organization, secondary metabolism, and glutathione metabolism potentially facilitate adventitious rooting in IBA-treated *Camellia sinensis*. Li et al. (2018) identified 4,294 differentially expressed genes (DEGs) and revealed that adventitious root formation in apple rootstocks is a complex process that depends primarily on the auxin signaling pathway. In addition, RNA-Seq is widely employed to characterize gene expression patterns and molecular mechanisms associated with lateral root initiation in maize (Jansen et al. 2013). This technique provides opportunities to elucidate the complex genetic backdrop and metabolic mechanisms of lateral root development and growth at the transcriptomic level.

In this study, various concentrations of NO (supplied by SNP) were used to investigate the functions of NO in lateral root development and growth of *K. obovata*. NO induced lateral root development and

growth in a dose-dependent manner. RNA-Seq was used to further explore the underlying regulatory mechanisms, and sets of DEGs associated with SNP addition were identified. Functional analysis of the DEGs indicated that NO mediates lateral root development by altering the expression of genes involved in starch and sucrose metabolism, hormone signal transduction, and cell wall metabolism.

Materials And Methods

Plant culture conditions and treatments

Propagules of *K. obovata* were collected from the Zhangjiang Estuary (23°55'N, 117°26'E) in Fujian Province, China. Healthy propagules of uniform size were planted randomly in pots (14 cm height, 12 cm diameter) filled with rinsed sand, with one propagule per pot. The optimum salinity for *K. obovata* growth at the sampling site is nearly 12‰ (Pan et al. 2018), and the hypocotyls were therefore grown with Hoagland solution containing 12‰ salinity. The pots were maintained in a greenhouse with 800–1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR), 60–70% relative humidity, and an air temperature of 25–28 °C (Chen et al. 2011, Chen et al. 2010).

SNP, frequently employed as an experimental tool with which to explore the effects of NO, was used as an exogenous NO donor (Shen et al. 2018). Propagules of similar size were divided into two groups. The first group was provided with Hoagland solution that contained 12‰ salinity and various amounts of SNP (0, 0.05, 0.1, 0.2, 0.5, 1, 5, 10 mM) in order to identify a suitable SNP concentration for use in subsequent experiments. In the second group, 200 μM FeSO_4 or $\text{K}_3[\text{Fe}(\text{CN})_6]$ were used as additional controls for SNP to discern the putative role of SNP derivatives (Shen et al. 2018). Besides, NO scavenger (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, cPTIO) was further used to clarify the role of NO in the lateral root development and growth of *K. obovata*. All solutions were replaced every 3 d to maintain constant concentrations (Chen et al. 2010). After 30 d, lateral roots of each treatment group were sampled and replicated three times. In addition, twenty plants were randomly mixed from each replicate as one sample, followed snap-frozen in liquid N_2 and stored at -80°C with the exception of some fresh roots that were used for morphology analysis with a Microtek ScanWizard EZ scanner and LA-S image analysis software (Hangzhou Wanshen Detection Technology Co., Ltd., China). Briefly, the collected fresh lateral roots were cleaned with distilled water. After wiping dry, roots of each treatment were placed in plastic container (30 cm $\times$ 48 cm) and scanned with Microtek ScanWizard EZ scanner. The lateral root length and surface area were subsequently analysis by LA-S image analysis software.

Determination of endogenous NO content

NO content was determined and calculated according to Kwan et al. (2015) by using the Griess Reagent System kit with three replicates according to the manufacturer's instructions.

RNA extraction and data processing

Lateral root samples from two treatments (0 mM SNP [CK] and 0.2 mM SNP [NO]) were fully ground in liquid N₂, and total RNA was extracted using an RNA isolation kit (Bioteke, China).

Transcriptome sequencing was performed on the Illumina NovaSeq platform. Clean reads were generated by trimming adapters, low-quality reads, and unknown bases (N). The clean, high-quality data were used for subsequent analyses. Transcriptome assembly was performed with Trinity (Grabherr et al. 2011). FPKM (fragment per kilobase transcriptome per million mapped reads) values and the DESeq2 R package were used to perform differential gene expression analysis (Love et al. 2014, Trapnell et al. 2010), and genes with $|\log_2 \text{Fold Change}| > 1$ and FDR-adjusted p -values < 0.05 were defined as DEGs. Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses were performed on the DEGs (Mao et al. 2005, Young et al. 2010). GO terms with corrected p -values < 0.05 were defined as significantly enriched and used to identify the primary biological functions of the DEGs. Likewise, a p -value < 0.05 was used to define significantly enriched pathways in the KEGG enrichment analysis.

Validation of RNA-Seq analysis by quantitative real-time PCR

Total RNA was extracted with an RNA isolation kit as described above. The cDNA was synthesized with a 5 × All-In-One RT MasterMix kit (Abm, Canada). Sequences of gene-specific PCR primers are provided in Supplementary Table S1. Gene expression was normalized to that of *18S*rRNA (GenBank accession: AY289625.1). For qRT-PCR, samples were analyzed in triplicate using a 20 μL PCR mixture that contained 2.0 μL cDNA, 0.4 μL primers, 10 μL 2 × ChamQ Universal SYBR qPCR Master Mix (Vazyme), and 7.2 μL ddH₂O. The amplification reaction conditions were 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, annealing at 60 °C for 30 s, and dissociation at 72 °C for 20 s. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

Statistical analysis

All data are presented as means $\pm$ SE, and statistical analyses were performed with SPSS software (one-way ANOVA, $p < 0.05$, SPSS for MAC, version 21).

Results

Responses of *K. obovata* roots to exogenous NO supply

First, we studied the ability of NO to regulate *K. obovata* lateral root development and growth by examining its impacts on root morphology. We tested various concentrations of the NO donor SNP in

order to identify a suitable concentration for subsequent investigations. As shown in Fig. 1, 0.05–1 mM SNP dramatically enhanced lateral root development and growth in *K. obovata* (Fig. 1A–D), and 0.2 mM was the optimal concentration. By contrast, lateral root development and growth were strongly inhibited at higher concentrations of 5 and 10 mM (Fig. 1).

FeSO₄ (Fe) and K₃[Fe(CN)₆] (CN) were used as additional controls to assess possible disturbances caused by SNP derivatives like Fe and ferricyanide. Besides, NO scavenger cPTIO was also applied as a negative control. Seedlings treated with 0.2 mM SNP had significantly greater root fresh weight, total root length, and root surface area compared with the CK. Moreover, Fe or CN treatment did not cause any significant alterations in these parameters (Fig. 2A–C). However, these effects were inhibited by cPTIO (Supplementary Fig. S1A–C). Besides, SNP application resulted in dramatically elevated NO contents in lateral roots compared with control. On the contrary, NO contents were decreased with the addition of NO scavenger cPTIO (Supplementary Fig. S2). These results indicate that NO itself, rather than other SNP derivatives, was responsible for enhanced lateral root development and growth in *K. obovata*. The 0 mM (CK) and 0.2 mM SNP (NO) treatments were adopted the RNA-Seq experiment.

***De novo* assembly and annotation of root transcriptomes**

We performed RNA-Seq analysis to investigate the molecular mechanisms by which exogenous NO modulates lateral root development and growth. Three independent biological replicates of the CK and NO treatments were used for paired-end sequencing. Information on the sequencing and assembly of the root transcriptomes is presented in Table 1. After filtering adapters and low-quality reads, we obtained 47,338,778 to 59,158,346 clean reads for individually sequenced libraries. The Q20 and Q30 values were 97.17–97.46% and 92.33–92.95%, and the GC contents were 45.59–45.75%. A set of 314.97 million clean reads was assembled with Trinity, resulting in the identification of 135,465 transcripts with an average length of 2,391 bp and 122,736 unigenes with an average length of 2,609 bp (Fig. 3A and B). The lengths of all transcripts and unigenes ranged from 201 to 17,241 bp (Fig. 3A and B). Of these unigenes, 110,839 (90.07%) were longer than 500 bp, 96,330 (78.40%) were longer than 1000 bp, and 68,328 (55.66%) were longer than 2000 bp (Fig. 3D). A total of 24,962 (20.34%) unigenes were annotated using the above-mentioned databases, and 109,049 (88.84%) unigenes received hits to at least one database, as shown in Table 2.

Functional classification of assembled unigenes

We performed a sequence similarity search to further discern the putative functions of the assembled unigenes. In total, 105,999 (86.36%) unigenes had significant hits to known proteins (Table 2). Among these unigenes, 37.7% and 50.2% had 60–80% and 80–95% similarity to their top BLASTX hit, and 5.7% and 6.4% had 40–60% and 95–100% similarity (Supplementary Fig. S3A). 45.7% of unigenes had BLASTX hits with *E*-values between 0 and 10⁻¹⁰⁰ (Supplementary Fig. S3B). The most frequent BLASTX

matches to our unigenes derived from *Hevea brasiliensis* (24.6%), *Jatropha curcas* (13.5%), *Manihot esculenta* (11.9%), *Populus trichocarpa* (11.7%), and *Ricinus communis* (8.9%). The remaining unigenes (24.6%) had top hits to sequences from other species (Supplementary Fig. S3C).

To further evaluate the putative functions of all unigenes, we performed Clusters of Orthologous Groups of proteins (COG), GO and KEGG annotation to categorize them and their encoded proteins into different functional categories. Twenty-five different categories were identified, comprehensively covering most biological activities (Supplementary Fig. S4). The largest fraction of unigenes was assigned to the general function category (14.4%). A large proportion of genes was also assigned to the posttranslational modification, protein turnover, and chaperones category (12.5%), followed by RNA processing and modification (7.82%), signal transduction mechanisms (7.81%), and translation, ribosomal structure, and biogenesis (7.62%) (Supplementary Fig. S4). GO analysis showed that 85,532 of the 105,999 unigenes were divided into 55 GO terms, of which cellular process, metabolic process, single-organism process, cell part, binding, and catalytic activity were the most common (Supplementary Fig. S5). In the pathway-based analysis, 21,388 unigenes (57.82%) were assigned to metabolism, 10,166 (27.48%) to genetic information processing, 2,283 (6.17%) to cellular processes, 1,611 (4.35%) to organismal systems, and 1,544 (4.17%) to environmental information processing (Supplementary Fig. S6).

GO and KEGG enrichment analysis

To identify candidate genes involved in NO-induced lateral root development and growth, gene expression patterns were compared between NO-treated and CK roots of *K. obovata* seedlings. A total of 1,593 DEGs were identified, 646 up-regulated (Fig. 4, red symbols) and 947 down-regulated (green symbols).

Functional enrichment analyses were performed to identify the putative molecular functions and biochemical pathways of the DEGs. GO enrichment analysis showed that genes up-regulated after exogenous NO addition were significantly enriched in GO terms related to carbohydrate metabolism, such as carbohydrate metabolic process (GO:0005975), disaccharide metabolic process (GO:0005984), glucan metabolic process (GO:0044042), and polysaccharide metabolic process (GO:0005976) (Fig. 5). By contrast, the down-regulated DEGs were significantly enriched in GO terms associated with protein metabolism, including protein phosphorylation (GO:0006468), protein modification by small protein conjugation (GO:0032446), and protein ubiquitination (GO:0016567). KEGG pathway enrichment analysis further indicated that starch and sucrose metabolism (ko00500), photosynthetic related metabolism (ko00860, ko00195, and ko00196), and ABC transporters (ko02010) were significantly enriched only in the up-regulated DEGs (Fig. 6). Phenylpropanoid biosynthesis (ko00940) and cyanoamino acid metabolism (ko00460) were enriched in both the up- and down-regulated gene sets. The down-regulated DEGs were enriched in biological processes mainly associated with plant-pathogen interactions and the biosynthesis of secondary metabolites. Taken together, our results suggest that starch and sucrose metabolism may be essential for NO regulation of lateral root development in *K. obovata*.

DEGs involved in starch and sucrose metabolism

Enrichment analysis showed that 16 DEGs (ten up- and six down-regulated) were involved in starch and sucrose metabolism (Fig. 7A). Of these, five of six genes encoding sucrose synthase (SUS), one gene encoding hexokinase (HK), two genes encoding trehalose 6-phosphate synthase (TPS), one gene encoding trehalose 6-phosphate phosphatase (TPP), and one gene encoding beta-amylase (BMY) were up-regulated. Three genes encoding cell wall invertase (CWIN), one gene encoding alpha-trehalase (treA), and one gene encoding glycogen phosphorylase (glgP) were down-regulated.

DEGs involved in plant hormone signal transduction

Sixteen DEGs were associated with plant hormone signal transduction (Fig. 7B). Among those associated with auxin signaling, eight of ten were down-regulated, including two of three genes encoding auxin/indole-3-acetic acid protein (Aux/IAA), three of four genes encoding auxin response factor (ARF), one gene encoding auxin-response factor SAUR36 (SAUR36), and one gene encoding GH3-auxin responsive promoter (GH3). Two genes encoding abscisic aldehyde oxidase (AAO) were involved in abscisic acid biosynthesis, and one gene encoding abscisic acid insensitive 5 (ABI5) was involved in abscisic acid signaling. One gene encoding 1-aminocyclopropane-1-carboxylate acid (ACC) synthase (ACS) and two genes encoding ACC oxidase (ACO) were involved in ethylene biosynthesis, and one gene encoding ethylene insensitive 2 (EIN2) was involved in ethylene signaling. In addition, five DEGs were associated with brassinosteroid (BR) signaling. Two DEGs were up-regulated, one encoding BR insensitive 1 (BRI1) and one encoding BR-signaling kinase (BSK). Two genes encoding BR resistant 1 (BZR1) and one gene encoding BR insensitive 2 (BIN2) were down-regulated.

DEGs involved in cell wall metabolism

Forty-two DEGs related to cell wall metabolism were differentially expressed after exogenous NO addition (Fig. 7C). Nineteen DEGs were associated with cellulose metabolism, including three genes encoding cellulose synthase A (CESA), two genes encoding COBAR-like protein (COBL4), one gene encoding endoglucanase 25 (KOR), and thirteen genes encoding beta-glucosidase (GLU). Six DEGs were associated with pectin metabolism, including one gene encoding galacturonosyltransferase 8 (GAUT8), two genes encoding pectinesterase (PME) and pectinesterase/pectinesterase inhibitor (PMEI), and one gene encoding pectate lyase (PL). In addition, four of seven genes involved in hemicellulose metabolism were up-regulated. Among them, two of three genes encoding xyloglucan endotransglucosylase/hydrolase (XTH), one gene encoding UDP-glucuronic acid decarboxylase 2 (UXS2), and one gene encoding xyloglucan glycosyltransferase 12 (CSLC12) were up-regulated. In addition, we also identified other genes involved in cell wall-related metabolism, such as three genes encoding expansin (EXP), three genes encoding L-ascorbate oxidase (AO), and single genes encoding leucine-rich repeat extensin-like protein 4

(LRX4), glucuronokinase 1 (CLcAK1), UDP-glucuronate:xylan alpha-glucuronosyltransferase 2 (GUX2), and UDP-arabinose 4-epimerase (MUR4).

Comparison of sequencing data and qRT-PCR results

To validate the RNA-Seq data, we performed qRT-PCR on 10 randomly selected genes. Gene expression patterns measured by qRT-PCR were consistent with those measured by RNA-Seq (Fig. 8A–J). In addition, there was a strong positive correlation ($R^2 = 0.8397$) between qRT-PCR data and FPKM values (Fig. 8K). Together, these results suggest that the sequencing data were reliable.

Discussion

Mangrove wetlands are influenced by chronic periodic tidal inundation, which is responsible for the typical characteristics of marsh habitats, such as abundant organic matter and nutrients and a reducing environment (Chen et al. 2010). Such wetlands serve as appropriate habitats for multiple microbes that contribute to the production of NO. We have previously investigated daily variations in NO emission flux in *K. obovata* wetlands (Chen et al. 2010). Based on this previous work, we designed the current study to unravel the effects of NO on root development and growth of a mangrove plant, *K. obovata*, using SNP as an exogenous NO donor. We found that NO promoted lateral root growth in a dose-dependent manner (Fig. 1). Under normal physiological conditions, NO is an important signaling molecule for a diverse range of organisms' physiological processes. However, excess NO produced in the organisms has obvious cytotoxicity. Now, various studies have been widely described its dual roles in the plant kingdom, which might depend on the cellular conditions and NO contents (Beligni and Lamattina 1999, Böhm et al. 2010). In soybean, high amounts of NO functioned as a stress factor resulting the decreasing of root growth that is correlated with cell death. Whereas lower concentration of NO, due to its action as a signaling molecule, could induce soybean root growth and lignification (Böhm et al. 2010). Studies also showed that the high concentration of NO accumulated in roots could effectively inhibit the root elongation and growth of tomato (Correa-Aragunde et al. 2004) and wheat (Groppa et al. 2008). In accordance with these results, some differences in the lateral root development and growth were found depending on the SNP concentration, the low concentrations (0.05–1 mM) stimulated the growth very effectively with the optimum concentration of 0.2 mM, while reverse influence was observed at higher SNP concentrations. However, few genomic resources were available for this genus. A *de novo* transcriptomic approach was therefore helpful for further investigating the changes in global gene expression that underlie modulation of *K. obovata* lateral root development by NO.

Changes in global gene expression induced by NO in *K. obovata*

Transcriptome sequencing was performed on the NovaSeq Illumina platform. After quality filtering, we assembled 122,736 unigenes from NO and CK libraries, and 105,999 of the unigenes showed significant similarity to known proteins in the NCBI Nr database (Table 2). The remaining 13.64% unannotated unigenes may be specific to *K. obovata* or may be homologous to genes with unknown biological functions in other species. Cellular process, metabolic process, single-organism process, cell part, binding and catalytic activity were the primary GO terms assigned to the unigenes, and these may be considered putative biological processes that respond to NO (Supplementary Fig. S5). This result is consistent with previously published transcriptomic data in which binding and catalytic activities predominated at the transcriptome level in response to NO in *Arabidopsis thaliana* (Hussain et al. 2016) and *Gossypium hirsutum* (Huang et al. 2018). KEGG pathway classification identified specific pathways involved in lateral root growth, such as transport and catabolism, signal transduction, translation, carbohydrate metabolism, and environmental adaptation (Supplementary Fig. S6).

Further functional enrichment analysis indicated that starch and sucrose metabolism was significantly up-regulated in response to NO (Figs. 5 and 6). Moreover, DEGs associated with plant hormone signal transduction and cell wall metabolism were also differentially regulated by NO (Fig. 7). Similarly, Li et al. (2016) suggested that sugar metabolism and the auxin and cytokinin signaling pathway primarily contributed to root development in grafted apple. GO enrichment analysis of birch cells treated with SNP also indicated that NO had a significant impact on carbohydrate metabolism and cell wall biosynthesis (Zeng et al. 2014).

The highly represented pathways and significantly enriched GO terms in our study suggested that NO positively enhances lateral root development and growth of *K. obovata* through the modulation of starch and sucrose metabolism, plant hormone signaling, and cell wall metabolism.

NO modulates genes associated with starch and sucrose metabolism to promote lateral root development and growth of *K. obovata*

Both internal and external factors impact the root growth of higher plants, and sugar is a particularly important internal factor (Ruan 2014). The mangrove plant *K. obovata* has a specific reproductive strategy known as vivipary (Chen et al. 1995). It has been demonstrated that hypocotyls of viviparous seedlings contain a considerable amount of starch that is used as an energy source during the early phase of development (Hanashiro et al. 2004). In this study, a key starch metabolic gene encoding β -amylase (BMY) was induced in response to exogenous NO, suggesting that NO may accelerate the process of starch hydrolysis, providing the necessary energy for lateral root development and growth.

Sucrose is the major form of carbohydrate translocated from photosynthetic sources to non-photosynthetic sinks such as the root system. When it reaches a sink, sucrose must be broken down into hexoses or their derivatives for use in multiple metabolic and biosynthetic processes (Ruan 2014). Invertase (INV) and sucrose synthase (SUS) are two major enzymes that are responsible for the cleavage of sucrose in higher plants (Ruan 2014). In this study, five of six *SUS* genes were up-regulated, whereas three *INV* genes encoding a typical cell wall invertase (CWIN) were down-regulated in *K. obovata* roots

after exogenous NO treatment (Fig. 7). Claeyssen and Rivoal (2007) demonstrated that CWIN activity coupled with a high hexose/sucrose ratio was commonly associated with high cell division rates during root initiation and expansion. Thereafter, a switch to the later maturation stage was accompanied by a shift from the INV to the SUS pathway of sucrose degradation (Claeyssen and Rivoal 2007, Weber et al. 1997). Increased SUS activity was associated with a transition from cell division to cell differentiation and elongation (Claeyssen and Rivoal 2007, Weber et al. 1997). We therefore inferred that NO may have a role in the promotion of lateral root elongation through up-regulation of *SUS* expression. This result is consistent with the changes in root morphology observed under exogenous NO addition (Fig. 1).

After the breakdown of sucrose into Glc and Frc, Frc can be irreversibly transformed into fructose 6-phosphate (F6P) by hexokinase (HK) (Galina and da Silva 2000). F6P may enter glycolysis and the tricarboxylic acid (TCA) cycle to produce energy, or it may be converted into UDP-Glc as a glucosyl donor for the synthesis of cell wall β -glucan (Galina and da Silva 2000). In this study, *HK* expression was markedly increased under exogenous NO addition, suggesting that NO may play a pivotal part in enhancing *K. obovata* lateral root development and growth by providing a consistent source of energy and cell wall polysaccharides.

Trehalose, a source of energy and carbon, can protect bioactive substances and cellular membranes from inactivation or denaturation under adverse stress conditions (Elbein et al. 2003). Trehalose biosynthesis in plants is a two-step pathway catalyzed by trehalose-6-phosphate (Tre6P) synthase (TPS) and Tre6P phosphatase (TPP) via Tre6P (Elbein et al. 2003). In this study, three genes associated with trehalose biosynthesis were up-regulated (two *TPS* and one *TPP*) in response to exogenous NO, whereas a gene encoding trehalase, which breaks down trehalose to form two Glc, was down-regulated. Trehalose metabolism has a fundamental and pervasive role in the life of plants (van Dijken et al. 2004). Overexpression of a Tre6P synthase/phosphatase fusion gene in rice elevated trehalose accumulation and conferred high tolerance to salt, drought, and low-temperature stresses (Garg et al. 2002). In addition, *AtTPS1* is central to normal vegetative growth and the switch to flowering in *Arabidopsis* (van Dijken et al. 2004). In the present study, expression of *TPS* and *TPP* was dramatically induced by NO, and that of *treA* was depressed, implying that trehalose biosynthesis is enhanced by exogenous NO addition and may contribute to *K. obovata* lateral root development and growth. Taken together, our results suggest that starch and sucrose metabolism is accelerated by NO, thereby supplying more energy to promote lateral root development of *K. obovata*.

NO modulates genes associated with plant hormone signal transduction to promote lateral root development and growth of *K. obovata*

Lateral root development and growth—including cell division, differentiation, expansion, and patterning—are tightly modulated by phytohormones (Bao et al. 2004, Ren and Gray 2015). One of the functions of auxin in plants is to regulate transcription by promoting the ubiquitination of auxin/indole-3-acetic acid (Aux/IAA) proteins through the activity of the SKP1-Cullin-F-box (SCF) complex and auxin transport inhibitor response1 (TIR1) or its paralog, auxin receptor F-box protein (ABF) (Deng et al. 2012). The auxin

response factors (ARFs) released from the degradation of Aux/IAAs serve as transcriptional repressors or activators of specific genes that contain the auxin-responsive element (AuxRE) promoter element (de Jong et al. 2015, Waller et al. 2002).

In this study, five auxin response genes (*IAA16*, *IAA27*, *AUX22*, *SAUR36*, and *GH3*) and four auxin response factor genes including one *ARF1* and three *ARF9* were differentially expressed in the roots of *K. obovata* in response to exogenous NO (Fig. 7). The primary auxin response genes can be classified into three major groups: Aux/IAAs, SAURs (SMALL AUXIN UP RNA) and GH3s (Gretchen Hagen3). Aux/IAA proteins are transcriptional repressors, and *S/IAA15* down-regulated tomato lines showed increased lateral root formation (Deng et al. 2012). GH3 family proteins promote the conversion of active IAA to its inactive form (Yang et al. 2015). Here, four primary auxin response genes (*IAA27*, *AUX22*, *SAUR36*, and *GH3*) were differentially expressed in response to exogenous NO, suggesting that the establishment of an appropriate auxin response system is important for NO's mediation of lateral root development and growth in *K. obovata*.

BRs are linked to lateral root development and interact with auxin to increase lateral root formation in *Arabidopsis* (Bao et al. 2004). It was well-known that the BR signal is perceived by the plasma-membrane-localized receptor kinase BRI1 and proceeds through the co-receptor BAK1 and additional downstream positive and negative regulators to mediate the expression of BZR1 and its homolog, BES1, which directly control BR-responsive gene expression (Chung and Choe 2013, Ye et al. 2011). Our results indicated that NO takes role in the BR signaling pathway to modulate root development and growth in *K. obovata*. The BR receptor kinase BRI1 and the positive regulator BSK were significantly up-regulated, whereas the negative regulator BIN2 and the transcription factors BZI1 and BES1 were significantly down-regulated. Combined with previous studies (Chung and Choe 2013, Ye et al. 2011), our results suggest that NO may promote lateral root development and growth by enhancing the BR perception and signaling pathway.

The action of ethylene in lateral root formation has previously been characterized using mutants in *Arabidopsis* (Alonso and Stepanova 2004, Negi et al. 2008). In the present study, one gene encoding ACC synthase (ACS) and two genes encoding ACC oxidase (ACO) were significantly down-regulated in response to exogenous NO (Fig. 7). These genes are involved in the two committed steps of the ethylene biosynthesis pathway. Initially, S-adenosyl-L-methionine (SAM) is transformed by ACS into ACC, in what is generally considered the rate-limiting step. Ethylene is then released from ACC by ACO (Park et al. 2018). Previous work has shown that the *aco1-1 Arabidopsis* mutant has reduced ethylene production in root tips and enhanced lateral root development compared to the wild type (Park et al. 2018). Our results suggest that NO influences ethylene synthesis, potentially affecting lateral root development through the suppression of *ACS* and *ACO*. A gene encoding ethylene insensitive 2 (EIN2) was also significantly down-regulated in response to exogenous NO. EIN2 is an important signal transducer in the ethylene signaling pathway, and its functional deficiency in *Arabidopsis* gives rise to conspicuous ethylene insensitivity and a failure to display known ethylene responses (Miyata et al. 2013, Roman et al. 1995). *LjEIN2-1* and *LjEIN2-2* from *Lotus japonicus* together control ethylene signaling to suppress root growth and nodule formation (Miyata et al. 2013). Based on our data and previous work, we concluded that NO may

decrease the expression of *ACS*, *ACO* and *EIN2*, thereby reducing ethylene biosynthesis and signaling and enhancing lateral root development and growth.

Accumulated evidence suggests that abscisic acid (ABA), a universal stress hormone, takes part in the regulation of lateral root development (De Smet et al. 2003, Xing et al. 2016). Two genes encoding abscisic aldehyde oxidase (AAO) and abscisic acid insensitive 5 (ABI5) were identified in this study (Fig. 7), both are associated with ABA synthesis and signaling. AAO functions in the final step of ABA biosynthesis by oxidizing ABA aldehyde to ABA (Seo et al. 2000). ABI5, a basic leucine zipper transcription factor, acts as a molecular hub in the NO-mediated balance between early development and stress (Albertos et al. 2015). NO facilitates seed germination in *Arabidopsis* through a mechanism linked to ABI5 degradation (Albertos et al. 2015). Taken together, these results suggest that decreased ABA levels and expression of *ABI5* mediated by NO may contribute to *K. obovata* lateral root development and growth.

NO modulates expression of genes associated with cell wall metabolism to promote *K. obovata* lateral root development and growth

Plant cell growth is restricted by the cell walls, dynamic and complex structures that consist of polysaccharides (mainly cellulose, hemicellulose, and pectin), highly glycosylated proteins, and lignin (Somerville et al. 2004). In the present study, exogenous NO altered the expression of 42 DEGs associated with cell wall biosynthesis and modification (Fig. 7).

As the major component of plant cell walls, cellulose is necessary for plant morphogenesis. Cellulose synthase (CESA) was first identified in cotton fibers (Pear et al. 1996), and its central role in the biosynthesis of crystalline cellulose was confirmed using the *rsw1* mutant of *Arabidopsis* (Arioli et al. 1998). Decreased CESA in primary walls is correlated with inhibited cell elongation and was revealed in some of these mutants to be associated with elevated levels of ethylene and jasmonate (Ellis et al. 2002). Genetic studies have identified a number of genes that contribute to the overall process of cellulose biosynthesis, including genes that encode endoglucanase 25 (KOR) and COBAR-like protein (COBL). KOR participates in cell wall assembly during the processes of cell plate maturation and cell elongation in cytokinesis, and it is necessary for the formation of cellulose microfibrils and the secondary cell wall (SCW) in the developing xylem (Zuo et al. 2000). *COBL*, encoding a glycosyl-phosphatidyl inositol-anchored protein, is primarily responsible for SCW biosynthesis. It affects cellulose crystallinity status and the orientation of cell expansion (Niu et al. 2015). A previous study revealed that *GhCOBL9A* and *GhCOBL13* are predominantly expressed during SCW biosynthesis in fiber development and are co-expressed with *GhCESA4*, *GhCESA7*, and *GhCESA8* in *Gossypium hirsutum* (Niu et al. 2015). In the present study, three *CESA*, two *COBL*, and one *KOR* gene were up-regulated in response to exogenous NO (Fig. 7), suggesting that NO may induce the expression of these genes to promote cell elongation and cellulose deposition during lateral root development and growth.

Xyloglucan is a polysaccharide that makes up about 20–25% (dry weight) of the primary cell wall in dicotyledons (Fry 1989). Xyloglucan endotransglucosylase/hydrolases (XTHs), key enzymes in

xyloglucan metabolism, promote cell expansion by catalyzing the cleavage of xyloglucan molecules and assembling new raw materials into the cell wall matrix (Pan et al. 2017). Two of three XTHs were significantly up-regulated in response to exogenous NO and may have promoted cell wall extension during lateral root development and growth. Other DEGs were also involved in hemicellulose metabolism, such as genes encoding cellulose synthase-like protein (CSL) and UDP-glucuronate:xylan α -glucuronosyltransferase 2 (GUX2) (Chou et al. 2015, Mortimer et al. 2010). *AtCSL* superfamily genes can be classified into six subfamilies and are thought to encode the catalytic subunits of enzymes that synthesize hemicellulose backbones (Chou et al. 2015, Richmond and Somerville 2000). Three genes encoding CSLs were differentially expressed in response to exogenous NO, indicating that NO may influence the biosynthesis of hemicellulose backbones.

Xylan, the principal hemicellulose in many plant secondary cell walls, has a backbone of β -(1,4)-linked xylosyl residues that is variably substituted with side chains, including methylglucuronic acid (MeGlcA) and glucuronic acid (GlcA) (Lee et al. 2012). GUX2, a xylan glucuronosyltransferase, is required in order to substitute the xylan backbone with MeGlcA (Mortimer et al. 2010). Deficiency of GlcA and MeGlcA side chains in the *gux1/2/3* triple mutant led to reduced secondary cell wall thickening and decreased plant growth in *Arabidopsis* (Lee et al. 2012). In the present study, *GUX2* was up-regulated in response to exogenous NO (Fig. 7), suggesting that NO may influence secondary cell wall deposition in *K. obovata* roots.

Pectin represents up to one-third of the cell wall dry mass and is crucial for the control of cell elongation (Hocq et al. 2017). Several DEGs were associated with pectin metabolism, such as a galacturonosyltransferase 8 gene (*GAUT8*), pectin methylesterase genes (*PMEs*), and pectin methylesterase inhibitor genes (*PMEIs*). GAUTs are pectin biosynthesis enzymes, and *GAUT4*-silenced tomato fruits have reduced starch accumulation and lower pectin levels, which contribute to greater fruit firmness (de Godoy et al. 2013). In this study, *GAUT8* was up-regulated in response to exogenous NO (Fig. 7), suggesting that NO treatment may alter pectin content and solubility in *K. obovata*. PME modifies cell walls by demethylesterification of the homogalacturonan (HG) backbone. PMEI, a specific proteinaceous inhibitor, is in charge of fine regulation of PME activity *in vivo* (Roeckel et al. 2008, Wolf et al. 2003). For example, Roeckel et al. (2008) reported that the interactions between PMEs and PMEIs are of vital importance to the cell wall stability of the tobacco pollen tube tip. In addition, inhibition of PME activity by two *Arabidopsis* PMEIs is a crucial means of controlling pectin esterification (Wolf et al. 2003). In our study, *PME* and *PMEI* were differentially expressed in response to exogenous NO (Fig. 7), suggesting that NO may affect the interaction between PME and PMEI, thereby promoting cell wall expansibility and leading to increased lateral root development and growth.

Additional DEGs were also involved in cell wall metabolism. These included genes encoding L-ascorbate oxidase (AO), leucine-rich repeat extensin-like protein 4 (LRX4), glucuronokinase 1 (GlcAK1), UDP-glucuronic acid decarboxylase 2 (UXS2) and UDP-arabinose 4-epimerase (MUR4). AO plays a significant role in redox maintenance and oxidative bursts in apoplasts, thereby controlling cell division and expansion (Xin et al. 2016). Two of three *AO* genes were up-regulated in response to exogenous NO (Fig.

7), suggesting that NO may promote AO activity and thereby modulate cell division and expansion in *K. obovata*. The importance of *LRX* for cell wall formation has been reported previously (Baumberger et al. 2003, Draeger et al. 2015). For example, *lrx1/lrx2* mutants showed impaired root hair cell wall structure and growth (Baumberger et al. 2003), and numerous alterations in the cell wall structure of *lrx3*, *lrx4*, and *lrx5* mutants confirmed the important role of LRX proteins in cell wall development (Draeger et al. 2015). In this study, *LRX4* expression increased in response to exogenous NO, suggesting that NO may mediate cell wall formation and development through its effects on *LRX4* expression. *A. thaliana* GlcAK is a key kinase that catalyzes the formation of UDP-GlcA and promotes the formation of cell wall polymers by supplying the required sugar donors (Pieslinger et al. 2010). *GlcAK* was up-regulated in response to exogenous NO, suggesting that NO positively regulates *GlcAK* expression, thereby promoting the synthesis of cell wall polymers for lateral root development and growth. UXS has been studied in a wide range of plants because of its vital role in sugar nucleotide interconversion and therefore in plant cell wall biosynthesis (Seifert 2004). The expression of *UXS2* increased markedly in response to exogenous NO, providing further evidence that NO influences cell wall biosynthesis in *K. obovata*. Arabinose (Ara) is an important constituent of various plant cell wall polymers and is essential to plant development and growth (Rautengarten et al. 2011). The *MUR4* mutant of *Arabidopsis*, which has a defective UDP-xylose 4-epimerase, exhibits impaired cell wall growth (Burget and Reiter 1999) associated with reduced synthesis of Ara-containing wall polymers. In the current study, *MUR4* expression was sharply elevated in response to NO, suggesting a potential role for NO in cell wall Ara biosynthesis. Taken together, our results demonstrate that NO is likely to play an important role in the metabolism of cellulose, hemicellulose, pectin and other cell wall components, thereby possibly promoting the root development and growth of *K. obovata*.

Conclusion

The role of NO in regulating the lateral root development and growth of a mangrove plant *K. obovata* was discerned in this study. We demonstrated that exogenous NO promoted *K. obovata* lateral root growth and morphology in a dose-dependent manner. Transcriptome data revealed that NO could confer more energy, modulate hormone signal transduction and cell wall metabolism-associated genes to accelerate the root development of *K. obovata*. Based on these findings, we propose a mechanism by which NO promotes lateral root development and growth in the mangrove plant *K. obovata* (Fig. 9).

Declarations

Author contributions

M. W., H. L. and H. Z. designed the experiments. M. W. and H. L. performed and analyzed the transcriptomic data. M. W. wrote the paper. Y. Z., C. G., D. M, Z. S, W. W, J. Z and Y. Y gave the suggestions. H. Z. revised this paper. All authors have read and approved the manuscript.

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Tables

Table 1 Summary of sequence assembly after Illumina sequencing. CK1-3: control plant without SNP addition; NO1-3: plant treated with 0.2 mM SNP.

Sample	Raw reads	Clean reads	Clean bases	Error (%)	Q20 (%)	Q30 (%)	GC (%)
CK1	55,456,022	54,843,800	8.23G	0.03	97.17	92.33	45.62
CK2	52,646,010	52,008,068	7.8G	0.03	97.31	92.60	45.64
CK3	47,931,948	47,338,778	7.1G	0.03	97.31	92.63	45.69
NO1	51,938,206	51,221,678	7.68G	0.03	97.29	92.57	45.59
NO2	50,989,436	50,398,246	7.56G	0.03	97.46	92.95	45.75
NO3	59,888,930	59,158,346	8.87G	0.03	97.41	92.88	45.68

Table 2 Summary of unigenes annotation against public available databases. Nr, NCBI non-redundant protein sequences; Nt, NCBI non-redundant nucleotide sequences; KO, Kyoto Encyclopedia of Genes and Genomes Ortholog; Swiss-Prot, a manually annotated and reviewed protein sequence database; KOG/COG, euKaryotic Ortholog Groups/Clusters of Orthologous Groups of proteins; Pfam, Protein family database.

Annotated public database	Number of unigenes	Percentage (%)
Annotated in NR	105,999	86.36
Annotated in NT	94,002	76.58
Annotated in KO	49,134	40.03
Annotated in SwissProt	88,209	71.86
Annotated in PFAM	85,532	69.68
Annotated in GO	85,532	69.68
Annotated in KOG/COG	39,489	32.17
Annotated in all Databases	24,962	20.33
Annotated in at least one Database	109,049	88.84
Total Unigenes	122,736	100

Figures

Figure 1

Effects of SNP concentrations (0-10 mM) on lateral root phenotype (A), average root fresh weight (B), root length (C), root surface area (D), and root number of *K. obovata* seedlings. Data are presented as means $\pm$ SE ($p < 0.05$).

Figure 2

Effects of SNP and its derivatives on average lateral root fresh weight (A), root length (B), root surface area (C), and root number of *K. obovata* seedlings. Data are presented as means ± SE (p < 0.05). CK: control plant without SNP addition, SNP: plant treated with 0.2 mM SNP, CN: plant treated with 0.2 mM K₃[Fe(CN)₆], Fe: plant treated with 0.2 mM FeSO₄.

Figure 3

Length distribution of assembled transcripts (A and C) and unigenes (B and D) from *K. obovata* seedling roots.

Figure 4

Volcano plot showing differential gene expression levels. Up- and down-regulated genes are represented by red and green symbols. Genes with no significant expression change are represented by blue symbols.

Figure 5

The most highly enriched GO terms of DEGs after NO treatment. The x-axis represents the number of DEGs in the enriched groups. The y-axis represents the different functional groups. The enriched GO terms of up-regulated DEGs are marked in red, and those of down-regulated DEGs are marked in green. Most of the up-regulated DEGs are enriched in carbohydrate metabolic process, whereas most of the down-regulated DEGs are enriched in protein metabolism.

Figure 6

KEGG enrichment analysis of DEGs after NO treatment. Enriched KEGG pathways of up-regulated DEGs (A) and down-regulated DEGs (B). Scale bar on the right represents the p-value. Starch and sucrose metabolism, photosynthetic related metabolism, and ABC transporters were significantly enriched only in up-regulated DEGs. Down-regulated DEGs were mainly enriched in the biosynthesis of secondary metabolites and plant-pathogen interaction.

A Starch and sucrose metabolism

Fold change (Log2)	Gene ID	Gene name
7.660	Cluster-8163.36910	sucrose synthase (SUS)
5.593	Cluster-8163.24987	sucrose synthase (SUS)
1.311	Cluster-8163.24988	sucrose synthase (SUS)
1.142	Cluster-8163.42294	sucrose synthase (SUS)
1.127	Cluster-8163.20165	sucrose synthase (SUS)
-4.600	Cluster-8163.57496	sucrose synthase (SUS)
-1.010	Cluster-8163.53984	cell wall invertase (CWIN)
-1.136	Cluster-8163.58472	cell wall invertase (CWIN)
-1.360	Cluster-8163.63549	cell wall invertase (CWIN)
9.340	Cluster-8163.80589	hexokinase (HK)
4.102	Cluster-8163.94619	trehalose 6-phosphate synthase (TPS)
1.947	Cluster-8163.24217	trehalose 6-phosphate synthase (TPS)
8.031	Cluster-8163.47232	trehalose 6-phosphate phosphatase (TPP)
-1.338	Cluster-8163.48264	alpha-trehalase (treA)
1.666	Cluster-8163.88885	beta-amylase (BMY)
-1.687	Cluster-8163.82291	glycogen phosphorylase (glgP)

B Hormone signal transduction

Auxin

Fold change (Log2)	Gene ID	Gene name
-6.029	Cluster-8163.62692	auxin response factor 9 (ARF9)
-10.122	Cluster-8163.62693	auxin response factor 9 (ARF9)
-6.937	Cluster-8163.67795	auxin response factor 9 (ARF9)
5.313	Cluster-8163.67283	auxin response factor 1 (ARF1)
1.237	Cluster-8163.49306	auxin-responsive protein IAA16 (IAA16)
-1.126	Cluster-8163.70696	auxin-responsive protein IAA27 (IAA27)
-1.990	Cluster-8163.66319	auxin-induced protein AUX22 (AUX22)
-1.227	Cluster-8163.19327	auxin-responsive protein SAUR36 (SAUR36)
-1.090	Cluster-8163.24699	GH3 auxin-responsive promoter (GH3)

Brassinosteroid

Fold change (Log2)	Gene ID	Gene name
1.468	Cluster-8163.100216	brassinosteroid insensitive 1 (BRI1)
1.475	Cluster-8163.34293	BR-signaling kinase (BSK)
-1.132	Cluster-8163.42773	brassinosteroid resistant 1 (BZR1)
-1.398	Cluster-8163.42775	brassinosteroid resistant 1 (BZR1)
-8.459	Cluster-8163.75120	brassinosteroid insensitive 2 (BIN2)

Ethylene

Fold change (Log2)	Gene ID	Gene name
-1.977	Cluster-8163.57046	1-aminocyclopropane-1-carboxylate acid synthase (ACS)
-1.411	Cluster-8163.56773	1-aminocyclopropane-1-carboxylate acid oxidase (ACO)
-1.900	Cluster-8163.54967	1-aminocyclopropane-1-carboxylate acid oxidase (ACO)
-9.891	Cluster-8163.50709	ethylene insensitive 2 (EIN2)

Absciscic acid

Fold change (Log2)	Gene ID	Gene name
-1.052	Cluster-8163.62514	abscisic aldehyde oxidase (AAO)
-5.663	Cluster-8163.98107	abscisic acid insensitive 5 (ABI5)

C Cell wall metabolism

Fold change (Log2)	Gene ID	Gene name
2.309	Cluster-8163.46547	cellulose synthase A (CESA)
1.040	Cluster-8163.51414	cellulose synthase A (CESA)
1.899	Cluster-8163.66821	cellulose synthase A (CESA)
3.696	Cluster-8163.12178	COBRA-like protein 4 (COBL4)
1.235	Cluster-8163.32513	COBRA-like protein 4 (COBL4)
7.281	Cluster-8163.65728	endoglucanase 25 (KOR)
1.901	Cluster-8163.79844	xyloglucan endotransglucosylase/hydrolase (XTH)
1.064	Cluster-8163.88753	xyloglucan endotransglucosylase/hydrolase (XTH)
-2.655	Cluster-8163.61646	xyloglucan endotransglucosylase/hydrolase (XTH)
1.070	Cluster-8163.61039	xyloglucan glycosyltransferase 12 (CSLC12)
-1.091	Cluster-8163.64650	cellulose synthase-like protein E2 (CSLE2)
-1.090	Cluster-8163.83451	cellulose synthase-like protein E3 (CSLE3)
1.563	Cluster-8163.98067	UDP-glucuronate:xylan alpha-glucuronosyltransferase 2 (GUX2)
2.048	Cluster-8163.77383	galacturonosyltransferase 8 (GAUT8)
1.933	Cluster-8163.48958	pectinesterase/pectinesterase inhibitor (PMEI)
1.586	Cluster-8163.48960	pectinesterase/pectinesterase inhibitor (PMEI)
-1.082	Cluster-8163.47132	pectinesterase (PME)
-2.101	Cluster-8163.52999	pectinesterase (PME)
-1.552	Cluster-8163.47457	pectate lyase (PL)
6.567	Cluster-8163.90167	L-ascorbate oxidase (AO)
1.201	Cluster-8163.58899	L-ascorbate oxidase (AO)
-6.458	Cluster-8163.51765	L-ascorbate oxidase (AO)
-1.560	Cluster-8163.45000	Expansin-A1 (EXPA1)
-2.090	Cluster-8163.45514	Expansin-A8 (EXPA8)
-2.938	Cluster-8163.16199	Expansin-A4 (EXPA4)
6.844	Cluster-8163.59325	Glucuronokinase 1 (GLCAK1)
1.821	Cluster-8163.44205	UDP-glucuronic acid decarboxylase 2 (UXS2)
1.171	Cluster-8163.63897	leucine-rich repeat extensin-like protein 4 (LRX4)
1.046	Cluster-8163.41441	UDP-arabinose 4-epimerase (MUR4)
6.260	Cluster-10087.1	beta-glucosidase (GLU)
3.440	Cluster-8163.61304	beta-glucosidase (GLU)
1.518	Cluster-8163.61889	beta-glucosidase (GLU)
-1.491	Cluster-8163.58961	beta-glucosidase (GLU)
-1.504	Cluster-8163.59381	beta-glucosidase (GLU)
-1.706	Cluster-8163.56559	beta-glucosidase (GLU)
-1.725	Cluster-8163.65154	beta-glucosidase (GLU)
-1.738	Cluster-8163.48332	beta-glucosidase (GLU)
-2.017	Cluster-8163.60799	beta-glucosidase (GLU)
-2.232	Cluster-8163.59377	beta-glucosidase (GLU)
-2.299	Cluster-8163.65954	beta-glucosidase (GLU)
-3.487	Cluster-8163.57597	beta-glucosidase (GLU)
-7.183	Cluster-8163.28391	beta-glucosidase (GLU)

Figure 7

Selected genes related to starch and sucrose metabolism (A), hormone signal transduction (B), and cell wall metabolism (C). The up- or down-regulated DEGs are represented in red or green. The color bar represents the intensity of gene expression.

Figure 8

Transcriptional expression levels of ten candidate genes measured by qRT-PCR and RNA-seq in the seedling roots of *K. obovata* treated with or without NO donor. A: *XTH*, xyloglucan endotransglucosylase/hydrolase, B: *CESA*, cellulose synthase, C: *PMEI*, pectin methylesterase inhibitor, D: *PME*, pectin methylesterase, E: *PL*, pectate lyase, F: *AAO*, abscisic aldehyde oxidase, G: *ACS*, 1-

TPP, trehalose 6-phosphate phosphatase, TPS, trehalose 6-phosphate synthase, treA, alpha-trehalase, UXS2, UDP-glucuronic acid decarboxylase 2, XTH, xyloglucan endotransglucosylase/hydrolase.

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