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Research article

Transcriptome analysis reveals interplay between hormones, ROS metabolism and cell wall biosynthesis for drought-induced root growth in wheat

Monika Dalal[a,*], Sarika Sahu[b], Sneha Tiwari[a], Atmakuri R. Rao[b], Kishor Gaikwad[a]

[a] ICAR-National Research Centre on Plant Biotechnology, New Delhi, 110012, India
[b] ICAR-Indian Agricultural Statistics Research Institute, New Delhi, 110012, India

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ABSTRACT

The ability of roots to grow under drought stress is an adaptive trait for crop plants especially under rain fed and restricted irrigation regime. To unravel the molecular mechanism of drought induced-root growth, root transcriptomes of two wheat genotypes viz. Raj3765 and HD2329, with contrasting root growth under drought stress were analyzed. Drought stress significantly enhanced total root length in Raj3765 as compared to that of HD2329. RNA-seq analysis led to the identification of 2783 and 2638 differentially expressed genes (DEGs) in Raj3765 and HD2329, respectively, under drought stress as compared with non-stress conditions. Functional annotation, gene ontology and MapMan analysis of the DEGs revealed differential regulation of genes for pathways associated with root growth and stress tolerance. Drought stress significantly upregulated auxin receptor (*AFB2*) and ABA responsive transcription factors (*MYB78*, *WRKY18* and *GBF3*) in roots of Raj3765. Although certain genes for ethylene pathway were downregulated in both the genotypes, *ACC oxidase* and *2OG-Fe(II) oxygenase* were upregulated only in Raj3765 which might contribute to maintenance of a basal ethylene level to maintain root growth. Several genes related to cell wall biosynthesis and ROS metabolism were significantly upregulated in Raj3765. Genes related to gibberellic acid, jasmonic acid and phenylpropanoid pathways were downregulated in roots of both the genotypes under drought stress. Our analysis suggests that a coordinated yet complex interplay between hormones, cellular tolerance, cell wall synthesis and ROS metabolism are required for drought induced root growth in wheat.

1. Introduction

Wheat, a major staple food crop of the world, accounts for 20% of the calories consumed by humans. Among all commercially grown crops, it occupies largest land area (~220 million ha) with ~729 million tones of production (FAO stats, http://www.fao.org/faostat/). Although the potential yield of wheat is 7–8 t/ha, the average yields are only about 3 t/ha. Wheat crop suffers yield loss due to several biotic and abiotic stresses. Among these, drought stress is one of the major causes of lower average productivity in 66% of rain fed wheat cultivated across the world (Rosegrant et al., 2002).

Roots play a vital role in water and nutrient uptake from soil, and constitute an important drought avoidance mechanism in plants. By extracting the available moisture from soil layers, roots help maintain transpiration and the water status of the cells, thereby supporting physiological functions necessary for plant growth under drought stress leading to yield stability. Change in root system architecture and water uptake have been associated with biomass accumulation and increased yield in maize (Hammer et al., 2009). Deep rooting due to change in root angle contributes to drought-tolerance and higher yield (Uga et al., 2013) while higher nodal roots and root dry weight confers yield stability under alternate wetting and drying conditions in rice (Sandhu et al., 2017). Thus, genetic improvement of root traits is important for sustaining yield under drought stress conditions.

The ability of roots to grow under drought stress is an adaptive trait for crop plants especially under rain fed and restricted irrigation regime. In India and China, majority of wheat growing area is dependent on irrigation and stored soil moisture. With reducing fresh water resources and unpredictable rain fall, focus is on restricted or limited irrigation of crop to increase water use efficiency (Zhang et al., 2018). Under such scenario, efficient use of irrigated water in initial growth stage and then exploitation of stored soil moisture in later stages by

* Corresponding author.
E-mail addresses: monika@nrcpb.org (M. Dalal), sahusarikaiiita@gmail.com (S. Sahu), snehabiotech23@gmail.com (S. Tiwari), rao.cshl.work@gmail.com (A.R. Rao), kish2012@gmail.com (K. Gaikwad).

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stress induced root growth would be advantageous.

Root growth at low soil water potential has been reported in *Arabidopsis,* soybean and maize seedlings (Sharp et al., 2004; Sponchiado et al., 1989; van der Weele et al., 2000; Yamaguchi and Sharp, 2010). The kinematic analysis of the primary root at low water potential in maize revealed longitudinal expansion in region close to the apex but reduction in basal area expansion under decreasing water potential (Sharp et al., 1988). The growth response in roots is associated with increase in osmotic adjustment (Voetberg and Sharp, 1991), ABA (Sharp et al., 1994), cell wall extensibility and enhanced expansin activities (Wu et al., 1996). The transcriptomic analysis of maize and soybean primary root showed propensity of genes related to wall loosening, ABA and ethylene signaling and carbohydrate metabolism in elongation zones (Song et al., 2016; Spollen et al., 2008). The proteomic studies in maize and soybean primary roots at low water potential showed increase in reactive oxygen species (ROS) scavenging proteins and region specific regulation of cell wall proteins, phenylpropanoid metabolism and increase in ferritin proteins in the elongation zone (Yamaguchi et al., 2010; Zhu et al., 2006). The molecular mechanism of root growth under drought still remains unexplored in an important cereal crop such as wheat. Hence, we carried out comparative transcriptome analysis of wheat genotypes with contrasting root growth under soil moisture deficit. The RNA-seq analysis revealed novel insight in to drought-induced root growth in wheat.

2. Materials and methods

2.1. Plant material and drought stress treatment

Seeds of two bread wheat (*Triticum aestivum* L.) genotypes i.e. Raj3765 and HD2329 were germinated on moist germination paper. After 48 h of germination, four uniformly germinated seeds were planted in soil filled pots and 16 pots for each genotype were maintained. The seedlings were grown under well watered conditions for 25 days. After 25 days, drought stress was imposed by withholding water in eight pots each of Raj3765 and HD2329. Remaining eight pots each of Raj3765 and HD2329 were watered optimally and served as non-stress control. The whole experiment was conducted during wheat growing season in natural conditions. After 14 days of water withholding, the level of drought stress was quantified by measuring leaf relative water content (RWC) (Barrs and Weatherly, 1962) and soil moisture content (gravimetric method). For root growth analysis, the plants were gently removed from three pots per treatment per genotype. The roots were washed and scanned in root scanner and data on root traits such as total root length, surface area and diameter were measured using WinRhizo2013a software (Regent Instruments Canada Inc.). The statistical significance was identified by two-way Analysis of Variance (ANOVA).

For RNA isolation, roots from four individual plants in each pot were pooled as a biological replicate. Three biological replicates were collected for control and stress treatment for both the genotypes, frozen in liquid nitrogen and stored at −80 °C till further use.

2.2. Total RNA isolation and transcriptome sequencing

The total RNA was extracted from control and stressed root samples of both the genotypes using TRIzol method (Invitrogen). The total RNA was subjected to on-column DNase I treatment (Sigma-Aldrich) to remove genomic DNA contamination. The quantity and quality of RNA was checked by Nano Drop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and Agilent 2100 bioanalyzer respectively (Fig. S1). Good quality total RNA from three biological replicates were pooled together and used for RNA-seq library construction. The libraries were sequenced on Illumina HiSeq 1000 (Illumina, San Diego, USA) and paired end reads were generated. The RNA-seq data was deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRP133407) with accession number SAMN08604941 and SAMN08604942 for Raj3765 control and stress, respectively; SAMN08604943 and SAMN08604944 for HD2329 control and stress, respectively.

2.3. Quality check and assembly of the reads

The raw reads of all the four samples were submitted to fastqc for checking the quality. Trimmomatics (v0.32) was used with default parameters to remove adapter and low quality reads (Bolger et al., 2014). The clean and high quality reads (Fred score > 30) of all the samples were assembled *de novo* into unique transcripts using Trinity software (Grabherr et al., 2013). Following assembly, the counts of transcripts and the N50 were calculated. The cleaned reads were re-mapped to the assembled contigs using Bowtie (Langmead et al., 2009).

2.4. Differential gene expression analysis

The expression level of assembled transcript was estimated as FPKM (fragments per kilo base of exon per million mapped reads) using RSEM (version 3.2) (Li and Dewey, 2011). The differentially expressed genes (DEGs) from each sample were identified using Edge-R (Robinson et al., 2010) with parameters such as FDR (false discovery rate) < 0.01, p-value < 0.01 and fold change (log ratio) (< -2 for down regulated genes and > +2 for up regulated genes).

2.5. Annotation and enrichment analysis

For annotation, all unigenes were blast searched in NCBI non-redundant (Nr) protein database (http://www.ncbi.nlm.nih.gov) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg). Functional classification by gene ontologies (GO) of all unigenes was performed using WEGO software (wego.genomics.org.cn/). The significant enrichment analysis of gene ontology (GO) terms was carried out using agriGO v2.0 software (p-value < 0.01) (http://bioinfo.cau.edu.cn/agriGO/) (Tian et al., 2017). Metabolic pathways analysis was carried out with MapMan (ver 3.3.0) (Thimm et al., 2004).

2.6. Validation of RNA-Seq analysis by qRT-PCR

The validation of RNA-seq data was carried out in an independent experiment carried out under similar experimental set up in the wheat growing season. Drought was imposed 25 days after germination. Samples were collected 14 days after water withholding and stress level on 14th day was measured by leaf RWC. For RNA isolation, roots from four individual plants in each pot were pooled as one biological replicate. RNA was isolated by TRIzol (Invitrogen) following the manufacturer's instructions. The extracted RNA was subjected to on-column DNaseI (Sigma) treatment to obtain DNA-free RNA. The RNA was further checked for DNA contamination, if any. The cDNA was synthesized from 1 μg of total RNA using Superscript–III reverse transcriptase (Invitrogen, USA). qRT-PCR was performed using three independent biological replicates and three technical replicates of each biological replicate from control and stressed root cDNAs from both the genotypes.

For qRT-PCR analysis, the cDNA was diluted to 1:10 times and 1.0 μL of diluted cDNA was used as a template in a 10 μL reaction volume in Realplex^4 system (Eppendorf) containing 250 nM of primers and 5 μL of SYBR Premix (KAPA SYBR FAST qPCR kit). Expression data were normalized using endogenous control gene, Ta2291 (ADP-ribosylation factor) expression (Paolacci et al., 2009)). The sequences of the primers used in the qRT-PCR analysis are listed in (Table S1). Relative fold change in expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).