

Comparative transcriptome analysis of waterlogging induced adventitious root and control root of *Mentha arvensis*

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Research Article

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Abstract

Waterlogging a significant problem to plant growth in the areas facing the waterlogged condition of the soil. In our previous findings, *Mentha arvensis* was shown to adapt better in waterlogging conditions by initiating the early onset of adventitious root formation. In this study, we analysed the adventitious root's transcriptome and compared it with the control root. Analysis of differentially expressed genes in adventitious root in comparison with the control root were grouped into four functional categories, i.e., carbohydrate metabolism, antioxidant activity, hormonal regulation, and regulation of transcription factors that may majorly contribute to the development of adventitious roots. Differential expression of the upregulated and uniquely expressing twenty-four transcripts in adventitious roots was validated using q-RT PCR. The biochemical parameters total carbohydrate, total protein contents and antioxidant enzymes such as nitric oxide, catalase, and superoxide dismutase (SOD) activity were enhanced in the adventitious root. These identified transcripts upregulated or uniquely expressed in the adventitious root will be useful to understand their role in the waterlogging-induced emergence and development of adventitious roots, leading to tolerance towards waterlogging stress in plants.

Key Message

This study identified and validated the transcript expression analysis of waterlogging-induced adventitious root

in *Mentha arvensis*. These transcripts will be useful in understanding the adventitious root emergence and

development.

Introduction

Waterlogging and flooding impair plant growth and development by diminishing the oxygen (O₂) supply (Loreti, van Veen, & Perata, 2016). It negatively impacts agricultural land and deteriorates crop yield (Voesenek & Bailey-Serres, 2015). Waterlogging causes a reduction in the oxygen supply because of its slow diffusion rate, which ultimately damages the plants. Plants modulate various physiological and morphological changes like acquiring adventitious root formation, hypertrophied lenticels initiation (at the base of the stem), or establishment of aerenchyma during waterlogging (Voesenek & Bailey-Serres, 2015). The emergence of adventitious root is specific to species (Lorbiecke and Sauter 1999; Argus et al., 2015) and determined by the plant's growth phase and duration of waterlogging. The emergence of adventitious root (ARs) has been investigated in many species as a waterlogging response, including sunflower and some tomato cultivars (Wample and Reid, 1978; Vidoz et al., 2010), rice (Lorbiecke and Sauter 1999), *Rumex palustris* (Visser et al. 1996), Eucalyptus species (Argus et al. 2015), tamarack (Calvo-Polanco et al., 2012), bittersweet (Dawood et al., 2013; Zhang et al., 2007). ARs may originate from the hypocotyl or stem containing more aerenchyma than the primary roots and help exchange gas, water and nutrient

uptake (Steffens & Rasmussen, 2016). Aerenchyma enhances oxygen leakage into the neighbouring anaerobic soil, drastically providing tolerance to waterlogging. Plants have also developed suberin in the exodermis/hypodermis to prevent radial oxygen loss (Abiko et al. 2012). ARs may also deliver additional O₂ and carbohydrate sources (Rich, Ludwig, & Colmer, 2012).

Mentha arvensis, a perennial plant of the genus *Mentha* is widely popular worldwide because of its commercial value of menthol mint, but most cultivars of *Mentha* are susceptible to waterlogging stress and show a wilted, yellow, rotted phenotype under stress conditions. However, a few resistant species, like *Mentha aquatica* (Haddadi et al., 2016) and *M. arvensis* have been identified by Phukan et al., 2014 due to their ability to form adventitious roots as early as between the second to third day of waterlogging.

Transcriptome profiling analysis of the *M. arvensis* control root (primary root without waterlogging treatment) and adventitious root (after 8 days of waterlogging treatment) was adopted to study the genetic control of waterlogging tolerance and understanding the underlying genes and regulating pathways related to this process.

Plant hormones have a vital function in the development of AR by hormonal signalling cascade. Auxin and ethylene have already established their major role in AR formation (Druege et al. 2016). Other hormones like cytokinin, jasmonic acid and salicylic acid cross-talk are environmental signal responsive and spatiotemporally control AR development (Gonin et al. 2019). Earlier studies have implicated sugars and carbohydrates in the expansion and maturation of ARs. The role of sucrose in lateral root and adventitious root emergence in *Arabidopsis* under dark conditions has been studied (MacGregor et al. 2008; Takahashi et al. 2003). Qi et al. 2019 have demonstrated that the interaction linking sugars or carbohydrates with auxin regulates waterlogging-induced AR formation. Stress-responsive transcription factors (TFs) such as AP2/ERF, bHLH, bZIP, MYB, WRKY and NAC gene expression are altered during waterlogging. AP2/ERF gene is mainly involved in regulating the waterlogging responses. Some of the TFs of this family are upregulated or downregulated, suggesting its critical role in waterlogging (Teoh et al 2022).

Antioxidant enzymes like SOD, and catalase have established the cross-talk between the phytohormones and ROS, as ethylene and auxin induce the AR formation through ROS signalling (Qi et al. 2019). Change in total carbohydrate and protein content during waterlogging stress indicates its regulation in AR formation.

Molecular mechanism and gene regulation are not much studied in how carbohydrates and the hormone signalling mediate the waterlogging tolerance by developing adventitious root to *Mentha arvensis* plant. So, in our current study, high-throughput de novo transcriptome scrutiny was carried out to yield a primary knowledge of the molecular mechanism of adventitious root initiation and waterlogging tolerance in *M. arvensis*. This study will lay the foundation for identifying important genes involved in forming adventitious roots during waterlogging conditions in this medicinally important plant, which can be

further utilized for better adaptability of waterlogging sensitive plants against waterlogging stress conditions.

Materials and Methods

2.1 Plant material and sampling:

5–6 week-old *Mentha arvensis* CIM-KOSI cuttings were planted in a pot filled with soil and allowed to get acclimatized. *M. arvensis* (CIM-KOSI) plants were grown according to Phukan et al., 2014 in the greenhouse chamber of CSIR-CIMAP Lucknow. For waterlogging treatment, plants were kept in bigger pots; the water level touches the plant 2–3 cm beyond the plant-soil interface. Root tissue samples of two independent biological replicates of the control and adventitious root were collected after 8 days of waterlogging treatment. They were preserved at – 80°C until use. These plant specimens were further utilized for RNA extraction and de novo sequencing, cross-section microscopy examination, antioxidant enzyme assay, total protein content and carbohydrate treatment.

2.2 Anatomical study of adventitious root and control root using safranin O staining:

For cross-section examination, one-month-old *M. arvensis* planted at the same time, control roots (Primary root) and adventitious roots originated, followed by eight days of waterlogging treatment, were harvested simultaneously. Roots were washed, and sections were cut manually using a razor; the middle part of the control and adventitious roots were stained with a drop of safranin-O (diluted 1:5 times with water) and covered for 2 minutes. Safranin was removed with filter paper, and the section was washed thoroughly using 70% ethanol for 1–2 minutes. The slide was fixed with 1–2 drops of 20% glycerol solution and was examined under the light microscope (OLYMPUS) at low power 2.5X objective lens.

2.3 Isolation of RNA and cDNA library preparation

RNA extraction was performed according to Isol-RNA Lysis Reagent Manual (© 2007 5 PRIME). Illumina-compatible SureSelect strand-specific RNA Library Prep kit (Agilent, Santa Clara, CA, U.S.A.) was utilized to prepare RNA sequencing libraries at the Genotypic Technology Pvt. Ltd., Bangalore, India. Briefly, enrichment of mRNA was performed from 500ng of Qubit quantified total RNA. The refined mRNA was subjected to fragmentation for 2 minutes at 94°C and manifested with divalent cations. The first strand of cDNA synthesis was performed along with Actinomycin D (Gibco, life technologies, Carlsbad, CA, U.S.A.). cDNA was purified using HighPrep magnetic beads (Magbio Genomics Inc, USA). Purified single-stranded cDNA synthesized the second strand, followed by end repair using the Second Strand Synthesis + End Repair mix. HighPrep magnetic bead was used for purifying end-repaired cDNA. Adapter ligation on cDNA was done after 3' Adenylation. HighPrep beads were utilized to purify the adapter-ligated DNA, and with the help of Illumina-compatible indexing primers, amplification for 10 cycles was kept utilizing the SureSelect Strand-Specific RNA Library Prep kit. The amplified product (sequencing library) was refined with HighPrep beads and subjected to superior-library control validation. Qubit fluorometer was initially

used 2 to quantify the sequencing library (Thermo Fisher Scientific, MA, USA), and the size of its fragment distribution was examined on Agilent TapeStation. Lastly, Kapa Library Quantification Kit (Kapa Biosystems, Wilmington, MA, USA) was used to estimate the sequencing library by quantitative PCR accurately. The final multiplexed library was created using equimolar amounts of qPCR quantified libraries for sequencing on an Illumina sequencer for 150 bp paired-end chemistry

2.4 De Novo Transcriptome Assembly by Illumina NextSeq500 Paired-end sequencing:

Reads were assembled using Trinity2 (default k-mers, i.e., 25. Trinity) to delineate a novel technique for the systematic and vigorous de novo transcriptome modification from RNA-seq data. Trinity separates RNA-seq into indefinite independent de Bruijn graphs; one expressed gene is represented by one individual graph. After this, parallel computing was done to restore transcripts, which comprehend alternatively spliced gene forms from their respective de Bruijn graph. There are three stages of Trinity: Inchworm, Chrysalis and Butterfly. Inchworm generates full-length transcripts into the unique sequence of transcripts but reveals the specific portions of alternatively spliced form transcripts. Inchworm often generates full-length transcripts for a dominant isoform but outlines the particular portions of alternatively spliced transcripts. It works well for datasets mostly lacking repetitive sequences, such as transcriptomes. Next, Chrysalis groups the related Inchworm contigs and builds de Bruijn graphs for every cluster. Each group exhibits the full transcriptional entangled for specific genes (or sets of genes with common sequences). Chrysalis then segregate the inchworm contigs and RNA seq reads into read into disjoint graphs. At last, Butterfly processes each graph in parallel, eventually reporting full-length transcripts of alternatively spliced form apart to paralogous genes. Through the graph, Butterfly traces the reads and figures out the association based on read sequences and upholds any valid paired-end data. Trinity spawn assembly is clustered, hingeing on the sequence similarity. CD-HIT3 version 4.5.4 4 (Cluster Database at High Identity with Tolerance) diminishes the redundancy lacking elimination of sequence multiplicity employed for additional transcript annotation for the differential gene expression. Tissue-specific unigenes were spawned by integrating the root samples from SO_6521 and SO_3233; similarly, adventitious root unigenes were generated using CD-HIT. The assembled transcripts from the two independent biological replicates with their significant *P values* were submitted to the SRA database of NCBI (SRA under the BioProject ID PRJNA546076).

2.5 Functional annotation of assembled reads:

According to Gene Ontology (GO) and biological pathway, assembled unigenes were grouped and annotated by mapping them to public databases, including NCBI non-redundant (NR), SwissProt, UniProt and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. After functional annotation using gene ontology attributes, transcripts were grouped into 3 GO domains i.e., cellular components, biological process and molecular function. Tissue-specific transcripts were annotated by the homology search against *Viridi plantae* data set from the UniProt database using BLAST-2.5.03. Primary and secondary metabolic pathway operations were accomplished corresponding to KEGG pathway database.

2.6 Differential gene expression analysis:

Master transcripts (unigenes) depicting control root and adventitious root tissue were created by clustering through CD-HIT. Read counts for all samples were generated by aligning reads to the unigene using bowtie⁶. Sequencing bias among the samples and differential analysis was carried out using DESeq⁷. We identified differentially expressed transcripts in adventitious and control roots with a significant P-value ≤ 0.05 cutoff/FDR of two-fold in control vs adventitious root upregulated, whereas five fold in specifically upregulated in the adventitious root. RPKM (Reads Per kb per Million reads) based cluster for all genes among two independent biological replicates. The transcript ID of differentially expressed transcripts in the adventitious root compared to the control root and transcripts specifically present in the adventitious root selected for our study is summarized in Supporting Information Table S1 and S2, respectively.

2.7 qRT-PCR analysis:

High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) was utilized to reverse transcribe 1 µg of purified RNA. The qPCR reactions were performed in 20 µl that comprised 2 µl of cDNA, 10 µl 2×SYBR Green Master mix (TaKaRa), and 0.4 µl of forward and reverse primer. Relative quantification and validation of transcripts identified from transcriptome data were analyzed by Applied Biosystems 7900 HT Fast Real-Time PCR machine using SYBR Green PCR master mix kit (TAKARA) and qRT-PCR reaction condition included nascent step (50°C for 2 minutes, 95°C for 10 minutes), 40 cycles (95°C for 15 seconds, 60°C for 1 minute) and denaturing phase (95°C for 15 seconds, 60°C for 15 seconds, 95°C for 15 seconds, 37°C for 2.5 minutes). As an endogenous control, actin was taken for normalization as it was constant across control and treated roots and was used for determining relative fold induction. Primer sets are listed in Table S3.

2.8 Identification of Transcription factor families:

In biological processes, transcription factors (TFs) are considered critical regulators at the transcriptional level of a gene (Riechmann et al., 2000). Transcripts deciphering the transcription factor in the sequencing of *M. arvensis* control and adventitious root tissues predicted by exploring against all transcription factor (TF) protein sequences dataset downloaded from PlnTFDB (<http://plntfdb.bio.uni-potsdam.de/v3.0/downloads.php>) using BLAST-2.5.0 with an e-value cut-off of 1.0e205.

2.9 Estimation of NO and antioxidants scavenging activity:

The study of enzymatic cellular antioxidants, whole plant under waterlogged and control conditions, was homogenized according to Gegenheimer P, 1990. The homogenate was centrifuged, and the supernatant was used for enzymatic activity.

2.10 Quantification of NO scavenging activity:

NO scavenging activity was performed according to Patel RM and Patel NJ, 2011, using the extract obtained above. To the test sample of volume 50 µl dissolved in DMSO, added 200 µl of 10mM sodium

nitroprusside followed by incubation for 2 hours at room temperature. Taken 50 ul of sample and 100 ul of Griess reagent were added. The O.D of the chromophore was recorded at 546 nm.

NO scavenging was determined by employing the formula:

$$\%Scavenging = \frac{Absorbancecontrol - Absorbancesample}{Absorbancecontrol} \times 100$$

2.11 Catalase activity (CAT):

Catalase (EC 1.15.1.1) activity was measured by reduction of H₂O₂ level measured at 240 nm according to Aebi (1984). The reaction mixture consists of 20 ul enzyme extract in 50mM phosphate buffer pH 7. The reaction was accelerated by the incorporation of H₂O₂ till 15mM concentration. Its depletion is recorded for 30 seconds at 240 nm. Enzyme activity was calculated using the molar extinction coefficient for H₂O₂ (36 M⁻¹ cm⁻¹) and expressed in terms of nmol mg⁻¹ protein⁻¹ min⁻¹.

2.12 SOD quantification:

SOD (EC 1.15.1.1) activity was calculated according to Das K et al., 2000. The reaction mixture consists of 50mM sodium phosphate buffer (pH 7.5), 13mM methionine, 75mM NBT (nitro blue tetrazolium), 2mM riboflavin, 0.1 mM EDTA, 100 uL of enzyme extract. The reaction mixture was irradiated at 5000 lx light intensity for 15 minutes, and absorbance was measured at 560nm using the non-illuminated sample as blank. One unit of SOD activity was interpreted as the enzyme amount required for 50% inhibition of the NBT reaction.

Quantification of carbohydrates:

Total carbohydrate was estimated by the phenol-sulfuric acid method given by Dubois et al. 1956. Plant tissue of ~ 1g was hydrolyzed and extracted in 2.5N HCl by boiling for 3 hours, then allowed to cool down at room temperature and sodium carbonate was used for neutralization till the effervescence vanished. The final volume was made up to 100 ml, and 1 ml of sample 500 ul of 4% phenol and 2.5 mL 96% sulfuric acid was added. Absorbance was measured at 490nm. We have prepared a standard curve of glucose of varying concentration from 50ug/ml solution.

Protein estimation:

To determine total protein, ~ 1 g root tissues were homogenized in 0.1N NaOH, vortexed and incubated for 30 minutes at room temperature and centrifuged at 5000x g for 15 minutes; the supernatant was utilized for the protein determination. Protein concentration was determined according to the Bradford method, using bovine serum albumin (BSA) as a standard prepared at different concentrations. 1 ml of sample and 5 ml Bradford dye (1:4 diluted) were mixed properly, and the absorbance was recorded at 595 nm by UV-visible spectrophotometer.

Results

3.1 Adventitious root formation, Root anatomy of adventitious root and control root:

M. arvensis plants were waterlogged for different days. The adventitious root onset starts 2–3 days after waterlogging treatment. The adventitious root protrudes from the stem region and grows towards the water's surface for air absorption. The adventitious root developed after 4th and 8th days are shown in Fig S1.

We have tried to distinguish the anatomical difference by microscopic cross-section examination of the *M. arvensis* adventitious root and control root. The surface area of the adventitious root transverse section was more than the control root. It was also observed that aerenchyma formation was induced in both roots. The number of aerenchyma increased in the adventitious root, but their size decreased compared to the control root with a wider stele. Also, an additional endodermis layer is formed and a reduction in xylem and phloem number under waterlogging-induced adventitious root (Fig. 1a and 1b).

3.2 Antioxidant enzymes activity:

The antioxidant enzymes (NO, Catalase, SOD) activity was studied in adventitious root emerged after 4 days, 6 days, 8 days and 10 days of waterlogging and control root. The nitric oxide scavenging activity increases with the duration of waterlogging treatment in both the roots, but NO activity was found more in adventitious with the increasing days of waterlogging treatment (Fig. 2a). NO production is essential for root cortical cell death, leading to aerenchyma formation (Wany et al. 2017). As shown in Fig. 1, more number in aerenchyma formation in the adventitious root can be correlated with an increase in the NO scavenging activity. Similarly, the activity of antioxidant enzyme CAT and SOD increased with the duration of waterlogging in roots. However, the activity of enzymes was gradually increased in the adventitious root with the waterlogging period indicating their function in waterlogging tolerance (Fig. 2b, 2c).

3.3 Carbohydrate level during waterlogging:

Carbohydrate concentration in the adventitious root was more significant than in the control root after different days of waterlogging treatment. The amount of carbohydrates was maximum after ten days of waterlogging treatment in the adventitious root (Fig. 3a). This can be correlated with Qi et al. 2020 who reported that carbohydrates might be involved in the emergence and elongation of adventitious roots during waterlogging, as the genes related to sucrose and starch metabolism are enhanced.

3.4 Soluble protein content:

The total protein content increased with the duration of waterlogging treatment, mainly in the adventitious root, maximally induced after 8 days of waterlogging treatment (Fig. 3b). Previous study suggests waterlogging enhances gene expression, further increasing the formation of new proteins. An increase in the protein content during waterlogging may indicate the involvement of these anaerobic

proteins and enzymes in physiological development, and they may be associated with glycolytic and fermentation pathways (Gibbs and Greenway 2003).

3.2 Illumina sequencing and de novo assembly:

cDNA libraries constructed from two independent biological replicates of adventitious and control root RNA of *M. arvensis* were sequenced using the Illumina NextSeq500 paired-end sequencing platform. Illumina sequencing generated an average of 38.33 million raw reads in adventitious roots and 22.81 million raw reads in control root. Adaptor-containing sequences were removed after sequencing and screening based on quality and ambiguity scores, 25.325 million reads in adventitious root, and 20.95 million reads in control root were obtained. The strategies used for whole transcriptome sequencing and assembly are summarized in Supporting information Fig. S2. High stringency filtering was done for data quality improvement, which includes removing reads containing adaptor or vector sequences and eliminating reads having phred quality ≤ 20 . The raw data were submitted at the NCBI Sequence Read Archive under the BioProject ID PRJNA498199. Trinity assembler subjected to de novo assembly, progressed by utilizing next-generation short-read sequences. High-quality reads of tissue-specific clustering resulted in 219957 unique transcripts (average length of 686bp and N50 of 792bp) in the adventitious root and 162193 (average length of 810bp and N50 of 1012bp) in the control root sample. The size distribution of contigs and scaffolds, along with length distributions of unigenes (contigs) of adventitious and control roots, are given in Fig. S3 and S4.

3.3 Identification of differentially expressed transcripts in waterlogged stress:

The clean reads from both samples (adventitious and control roots) were compared to the unigenes library and differential gene expression was estimated using DESeq7, which uses a relative log expression approach by generating a virtual library in which all samples were compared against each other. A large number of DGEs were identified after comparing both samples. In differential gene expression data, a total of 208081 unigenes were found in which 47534 unigenes were upregulated, 36037 unigenes were downregulated and 124510 unigenes were neutrally regulated in the adventitious root. Transcripts present in both adventitious and control root samples but expressed more in adventitious root than control root with their expression values were shown as scattered plot analysis in Fig. 5. This analysis found that 73317 and 20351 unigenes were specific to the adventitious and control roots, respectively. Control versus adventitious root upregulated contains 3829 P value ≤ 0.05 unigenes log2fold change of that have whereas adventitious root specific contains 8121 unigenes with significant P value ≤ 0.05 . Among unigenes with significant P value in control versus adventitious root upregulated and unigenes specific to adventitious root, we have searched for unigenes that were found to be involved in hormonal regulation, carbohydrate metabolism, antioxidant activities, and regulation of transcription (Transcription factor). From transcriptome data, 41, 125, 37 and 75 upregulated transcripts were found in response to hormonal regulation, carbohydrate metabolism, antioxidant activities, and regulation of transcription, respectively, in adventitious roots after waterlogging treatment.

3.4 Upregulated transcripts related to hormonal regulation of adventitious root in waterlogged *M. arvensis*:

Plants hormones are vital endogenous regulators that provide the waterlogging responses via a signalling cascade mechanism by changing the balance between hormone synthesis and transport (Benschop et al., 2006; Wu et al. 2019; Yamauchi et al. 2020). Previous literature suggests ethylene is an essential waterlogging-responsive hormone, and ethylene is needed to form adventitious roots. The transcriptome-wide analysis showed that 12 transcripts of S-adenosylmethionine were accumulated specifically in the adventitious root. Based on differential gene expression 4 significant transcripts might be involved in auxin synthesis in control and adventitious root. Our transcriptome data also revealed the accumulation of ABA in adventitious roots (7 transcripts) during waterlogging. DEGs encoding 6 unigenes were upregulated in adventitious root suggesting the involvement of ABA signalling during waterlogging-induced adventitious root formation. DEGs encoding 12 cytokinin-related unigenes and 3 strigolactone-related transcripts were upregulated in the adventitious root, suggesting cytokinin signalling involvement during waterlogging-induced adventitious root formation (Fig. 6d and 7d).

3.6 Involvement of antioxidant system during waterlogging:

In plants, antioxidant defence mechanism enzymes are highly upregulated during waterlogging and provide tolerance. Antioxidant enzymes like SOD, CAT, PAT, APX in ROS scavenging and protect plants from damage. SOD is the first defence against ROS as it acts on superoxide free radicals (Alscher, Erturk & Heath, 2002). In the present sequencing data, 14 and 4 unigenes of SOD were found upregulated specifically in the adventitious root and upregulated in the adventitious root compared to the control root, respectively. Transcripts related to antioxidant enzymes like catalase (5), and glutathione peroxidase (9) were found in control vs adventitious root upregulated and specific to adventitious root (Fig. 6b and 7b).

3.7 Increased carbohydrate metabolism after waterlogging:

In our transcriptome data, carbohydrate metabolism-related genes involved in cellular metabolism, cell wall biosynthesis and respiration were upregulated after waterlogging treatment in the adventitious root. Transcripts related to carbohydrate metabolism that were upregulated in adventitious roots are pyruvate kinase (4 unigenes EC 2.7.1.40), glyceraldehyde-3-phosphate dehydrogenase (35 unigenes EC 1.2.1.), Glucose-6-phosphate 1-dehydrogenase (8 unigenes EC 1.1.1.49), phosphofructokinase (2 unigenes EC 2.7.1.19), Fructose-bisphosphate aldolase (8 unigenes EC 4.1.2.13), enolase (3 unigenes), NADH dehydrogenase (4 unigenes), Isocitrate dehydrogenase (5 unigenes EC 1.1.1.42), Pyruvate dehydrogenase E1 component (2 unigenes EC 1.2.4.1) and few other transcripts related to carbohydrate metabolism were found that signifies their involvement in energy regulation and regulation to stress tolerance in waterlogged *M. arvensis* roots (Fig. 6a & 7a).

3.8 Response of TFs to Waterlogging:

TFs are the key regulators in biotic and abiotic stress that bind to the *cis*-elements in the promoter region and modulate gene expression. In the present study, a total of 24508 (11.14%) transcripts in adventitious

root and 25202 (15.54%) transcripts in control root encoding transcription factors, belonging to 82 families were identified (Fig. S6). Members of C3H TF family were found to be most abundant (8.12% transcripts in adventitious root and 7.32% transcripts in control root) followed by FAR1 (6.88% transcripts in adventitious root and 7.14% transcripts in control root) and TRAF (5.10% transcripts in adventitious root and 5.98% transcripts in control root) families of transcription factors. Based on significant *P-value*, it was found that C3H (39), Orphans (33), mTERF (31), MYB-related (23), FAR1 (22), AP2 (21), bHLH (17) and bZIP (16) were upregulated in adventitious root as compared to the control root (Fig. 6c and 7d, Fig S7).

3.8 Validation of DGE patterns by qRT-PCR:

For the validation of the differential gene expression profile between the control root and adventitious root, we have selected 24 transcripts (upregulated in the adventitious root as compared to the control root and specific to the adventitious root) that may be related to adventitious root formation and regulation of waterlogging response in *M. arvensis* their expression was calculated after 4 days and 8 days of waterlogging treatment. Some transcripts related to transcription factors of family MYB, AP2/ERF, bZIP, ARF, bHLH) and carbohydrate metabolism were selected for validation by qRT PCR. Adventitious and control root samples of *M. arvensis* were harvested after 4 days, 8 days of waterlogging treatment and RNA was isolated. These selected transcripts showed differential accumulation in qRT-PCR after waterlogging treatment in the adventitious root compared to the control root (Fig. 8), further validating the NGS result. Transcripts maximally upregulated after 4 days of waterlogging treatment belong to ERF2 family TFs and its relative fold expression was more than 8-fold. Transcripts like cDOF, EIN, bZIP, MYB DRE, and MAD class of TF were upregulated by more than 2-fold after 4 days of waterlogging treatment. After 8 days of waterlogging treatment, most transcripts were more upregulated than those transcripts upregulated after 4 days of treatment. Transcripts like 9 cis-epoxy carotenoid dioxygenase, phosphoglycerate kinase, and auxin response factor (ARF) were maximally upregulated i.e., above 25-fold after 8 days of waterlogging treatment. EIN, GLK, DRE, sugar transporters (STP), and pyruvate kinase were upregulated by 15-fold and above. Other classes of TF's family, like bHLH, ERF, bZIP, EIN, TGA, MYB were found to be induced by 10-fold or above. CAT and another transcript of MYB TF was least expressed, i.e., by 5-fold or below (Fig. 8), indicating the involvement of these transcripts in waterlogging conditions.

Discussion

Waterlogging stress in plants evokes physiological, morphological and molecular level changes. Waterlogging conditions first influence the root; the adventitious root development and aerenchyma emergence during waterlogging stress is an essential adaptive trait. In this study, we have tried to understand the mechanism behind the waterlogging tolerance in *M. arvensis* by comparing the control root (primary tap root) and waterlogging-induced adventitious root (8 days of waterlogging treatment). The adventitious root contains a more significant number of aerenchyma that not only facilitates gas

transport from shoots to roots but also decreases the number of cells, which consumes more oxygen (Mano Y & Omori F. 2013).

Waterlogging results in extensive transcriptome profiling, and we investigated the differential gene expression pattern. After removing adaptor or vector-containing sequences, we obtained 25.325 million reads in the adventitious root and 20.95 million reads in the control root. After de novo assembly, we obtained 219957 and 162193 high-quality transcripts unique to the adventitious root and transcripts in the control root.

4.1 Transcriptome-based differential expression profiling provides a genetic basis for the adaptive response of plants after waterlogging:

In the context of global climate change, waterlogging emerges as a serious concern affecting the worldwide productivity of crops. Different cellular and metabolic changes occur inside the plant system to overcome this condition, which makes them necessary to understand to make them tolerant under these circumstances. Plants undergo morphological and anatomical changes during this stress period along with cellular level changes. For example, in cucumber, waterlogging induced the accumulation of ethylene, resulting in the formation of ARs (Qi et al. 2020). Aerenchyma emergence in ARs is an adaptable response resulting from programmed cell death regulated by hormones, mainly ethylene, triggered in many steps. Comparative RNA-seq-based transcriptome analysis between waterlogging tolerant and sensitive varieties of cucumber (Xu et al. 2017) and *Chrysanthemum morifolium* (Zhao et al. 2018) broadens our knowledge regarding the molecular mechanism of waterlogging response in plants. High-throughput RNA sequencing was also adopted for elucidating the functional pathway and regulatory gene network of the waterlogged root system of maize (Arora et al. 2017) and *Jatropha* (Juntawong et al. 2014). Comparative functional enrichment and gene ontology study of the root transcriptome of these species demonstrated the physiological response promoted by waterlogging treatment. In this study, we have made a comparative de novo transcriptome analysis of the control root vs 8th day waterlogging induced adventitious root of *M. arvensis*. This transcriptome analysis has helped to identify differentially regulated transcripts involved in waterlogging-induced adventitious root formation, which has not been extensively studied. DEG data shows among 208081 unigenes, 47534 unigenes were upregulated, 36037 unigenes were downregulated and 124510 unigenes were neutrally regulated in adventitious root. This indicates the differential gene expression responsive to waterlogging. Gene ontology enrichment analysis grouped transcripts into three domains biological process, cellular components and molecular functions (Fig. 4 and S5). Transcripts in response to hormonal regulation, carbohydrate metabolism, antioxidant activities, and regulation of transcription in adventitious roots were searched. Based on the previous literature, transcripts related to these metabolisms may be involved in providing waterlogging tolerance to the plant.

4.2 Regulation of hormone in waterlogged *M. arvensis* adventitious root emergence:

Ethylene is the primary factor that induces adventitious root formation during waterlogging stress (Sasidharan et al. 2015), and it was observed that ethylene biosynthetic inhibitor (CoCl_2) suppresses adventitious root formation in *M. arvensis* by inhibiting ACC oxidase, an important enzyme of the biosynthetic pathway of ethylene (Phukan et al. 2014). In the current study, twelve transcripts of S-adenosylmethionine, an intermediate of ethylene signalling, were explicitly accumulated in the adventitious root. Ethylene also regulates the hormone signalling of abscisic acid, gibberellic acid, and eventually auxin that, leads to adventitious root formation in tomatoes, *Solanum dulcamarum*, and *Oryza sativa* (Steffens et al. 2006; Dawood et al. 2016). Auxin accumulation in the plinth of the stem promotes adventitious root formation. It was observed that in the tomato plant, adventitious root formation depends upon auxin concentration, whereas in the tomato plant, in which auxin sensing is compromised, the emergence of the adventitious root is prohibited (Vidoz et al. 2010). There were 4 transcripts related to auxin present in the *M. arvensis* adventitious root. Our transcriptome data showed the accumulation of 7 ABA transcripts during the waterlogging; this can be correlated with ABA form increased extensively as the period of flooding progressed (Zhang et al. 1987). During waterlogging onset, plant roots promptly mediate xylem-borne stimulus to leaves via hormone, most prominently abscisic acid (ABA), which slows transcription via the complete closure of stomata (Jackson, 2005). In the pea (*pisum sativum*), after 4 days of flooding, it was observed that the abscisic acid (ABA) level was upregulated in the roots, resulting in complete stomatal closure.

In our study, transcripts belonging to the hormonal group of auxin, ethylene and abscisic acid biosynthesis, transport and metabolism were found to be upregulated in the adventitious root, indicating that these groups of hormone acts as a central regulator of waterlogging response and adventitious root formation. We have also found that 12 and 3 transcripts related to hormone biosynthesis and cascade were upregulated in adventitious roots related to cytokinin and strigolactone biosynthetic processes. Ethylene and abscisic acid-related transcripts were majorly found to be upregulated in the adventitious root, as it has already been reported their signalling cascade increases and implicates the emergence of the adventitious root (Vidoz et al., 2010) (Fig. 6d and 7d).

4.3 Effect of waterlogging on regulatory transcription factor:

Transcription factors (TFs) modification activity dynamically changes the transcriptome, which causes metabolic and phenotypic changes (Mitsuda et al. 2009). Transcriptional regulation in waterlogging conditions (hypoxic) has been extensively elucidated in the dicot model plant *Arabidopsis thaliana*. Transcription factor family of MYB, NAC, and AP2/ERF are shown to affect hypoxia responses in *Arabidopsis* (Hsu et al. 2011). As discussed earlier, ethylene has a significant role in adventitious root formation during waterlogging, and an ERF transcription factor regulates ethylene signal transduction (Licausi et al. 2013). The main transcription factor that regulates the hypoxia-related gene expression and responses to waterlogging conditions is Group VII ERFs (Mizoi et al. 2012). In *Jatropha*, JcERFVII-2 and JcERFVII-3, were two ethylene response factors (ERFs) that were found to induce in response to waterlogging (Juntawong et al. 2014). In addition to AP2/ERF, a MYB transcription factor of *Triticum*

aestivum i.e., TaMyb1 is vigorously induced in the roots under the hypoxic condition (Lee et al. 2007). In our data, most transcripts related to the transcription factor were involved and expressed in the adventitious root. The top five transcription factor families showing upregulated expression in adventitious root after waterlogging include C3H zinc finger, Orphan, mTERE, MYB related, FAR1, AP2/ERBP family of transcription factor. The determination of 54 families of transcription factors are either uniquely expressed or upregulated in adventitious root indicates a tight regulation and cross-talk of different signalling pathways during waterlogging.

4.4 Effect of waterlogging on antioxidative defence system:

Adventitious root growth is enhanced by both hormonal signals and reactive oxygen species. Ethylene by the plasma membrane-located NADPH oxidase enhances superoxide anion generation in the flooded rice. Accumulation of ROS augments root development by suppressing antioxidant enzyme catalase (with aminotriazole), whereas scavenging of hydrogen peroxide (with potassium iodide) suppresses the growth (Steffens et al., 2012), which indicates the hydrogen peroxide significance in crown root growth (Steffens et al., 2006). Some of the antioxidant enzymes involved in detoxification are superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidases (APX). Similarly, transcripts belonging to the catalase, peroxidase and superoxide dismutase were found to be upregulated in adventitious root in our transcriptome data, also after performing the biochemical activity followed by different days of waterlogging treatment antioxidant enzymes like catalase, SOD and NO scavenging activity was markedly induced in adventitious root in comparison with control root indicating that either they are involved in adventitious root development or plant is coping up under stressful environment after waterlogging treatment.

4.5 Waterlogging also affects energy-consuming metabolic processes:

The availability of carbohydrates, their metabolism and transport are necessary to regulate waterlogging and multiple abiotic stress tolerance (Rosa et al., 2009). In deep waterlogging conditions and if waterlogging persists long, the carbohydrate availability from the photosynthetic process or stored food reserve becomes crucial. Therefore, during the above-discussed waterlogging conditions, plants must alter the survival mechanism involving carbohydrate consumption (Bailey-Serres et al. 2008). It was reported that gene expression of α -amylase (OsAmy3D) in submerged rice leaves is modulated by SUB1C, an ethylene-responsive factor (ERF) (Fukao et al., 2006). Gibberellic acid positively regulates this gene and negatively by a related ERF SUB1A1. These findings suggest that carbohydrate levels in submerged plants are under hormone control. Glycolytic genes in *S. dulcamara* were enhanced after 6 h of submergence, demonstrating metabolic acclimatization to waterlogging stress (Dawood et al. 2013). Metabolic mechanisms related to carbohydrate mobilization in *Cucumis sativus* L. are related to its capacity to face oxygen deprivation and generate adventitious roots (Xu et al., 2018). Recent experiments suggest that flood-resistant and sensitive plants have different abilities to maintain their carbohydrate

content during waterlogging (Vu & Yelenosky 1991; Ferner et al. 2012). Plants which are more waterlogging sensitive their soluble sugar level deplete faster as compared to tolerant varieties as they were able to maintain a high level of total carbohydrates during the stress (Ferner et al. 2012); this correlates with our data where we found carbohydrate level was more in waterlogging induced adventitious root as compared to the control root (Fig. 3A). Reserve starch breakdown can partially fulfil the carbohydrate demand in the waterlogging tolerant species (Kreuzwieser et al. 2014). During waterlogging stress, carbohydrate metabolism is activated in the root system to produce more glucose and maintain energy levels (Zhang et al. 2017). Waterlogging-responsive AP2/ERF from *M. arvensis* MaRAP2-4 is shown to regulate *AtSWEET10* in *Arabidopsis thaliana*, leading to stress response (Phukan et al. 2018). In our transcriptome data, enzymes related to carbohydrate metabolism like pyruvate kinase, fructose 6- phosphotransferase, glyceraldehyde-3-phosphate dehydrogenase etc., were found to be upregulated in waterlogging-induced adventitious root along with that the total carbohydrate content of adventitious root is found to be higher than control root suggesting their involvement in energy generation process required during the adventitious root generation under waterlogging.

Conclusion

In conclusion, the present study has identified the transcripts that are either uniquely expressed in the waterlogging-induced adventitious root or upregulated in the adventitious root compared to the control root. The adventitious root shows higher nitric oxide scavenging activity, catalase and SOD activity, and total carbohydrate and protein amount was higher in the adventitious root than the control primary root. The selected transcripts were categorized into four categories (Carbohydrate metabolism, antioxidants, hormonal response and transcriptional regulation), and some were validated using qRT-PCR. This study provides a foundation for understanding the waterlogging-induced adventitious root formation in *M. arvensis*.

Abbreviations

GO, Gene ontology AR, Adventitious root, TFs Transcription factors

Declarations

Author contribution statement

S has performed the treatment in plants, analysis of sequences, biochemical analysis, real-time PCR and wrote the paper. DKP has performed root section cutting and staining. SU has helped in real-time analysis and writing of the manuscript. UJP has prepared the first replicate of the adventitious root sample. RKS has conceptualized the theme, planned the experiments, and edited the final manuscript. All authors finally read and approved the manuscript.

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Data availability

SRA Study accession number SUB5724447. The raw data were submitted at the NCBI Sequence Read Archive under the BioProject ID PRJNA546076. BioSample ID: SAMN11952060; SO_3233_MA_Control_Root: SAMN11952061: SO_3233: Adventitious root MA_8D_WL_Root SAMN11952062: SO_6521_MA_Control_Root SAMN11952063: Adventitious root SO_6521_MA_8D_WL_Root

Conflict of interest

The authors declare no conflict of interest.

References

1. Abiko T, Kotula L, Shiono K, Malik AI, Colmer TD, Nakazono M (2012) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zoizanthus* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. *mays*). *Plant Cell Environ* 35(9):1618–1630. 10.1111/j.1365-3040.2012.02513.x
2. Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53(372):1331–1341
3. Argus RE, Colmer TD, Grierson PF (2015) Early physiological flood tolerance is followed by slow post-flooding root recovery in the dryland riparian tree *Eucalyptus camaldulensis* subsp. *refulgens*. *Plant Cell Environ* 38(6):1189–1199. 10.1111/pce.12473
4. Arora K, Panda KK, Mittal S, Mallikarjuna MG, Rao AR, Dash PK (2017) RNAseq revealed the important gene pathways controlling adaptive mechanisms under waterlogged stress in maize. *Sci Rep* 7:10950. 10.1038/s41598-017-10561-1
5. Bailey-Serres J, Voesenek LA (2008) Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* 59:313–339. 10.1146/annurev.arplant.59.032607.092752
6. Benschop JJ, Bou J, Peeters AJ, Wagemaker N, Gühl K, Ward D, Hedden P, Moritz T, Voesenek LA (2006) Long-term submergence-induced elongation in *Rumex palustris* requires abscisic acid-dependent biosynthesis of gibberellin1. *Plant Physiol* 141(4):1644–1652. 10.1104/pp.106.082636
7. Calvo-Polanco M, Señorans J, Zwiazek JJ (2012) Role of adventitious roots in water relations of tamarack (*Larix laricina*) seedlings exposed to flooding. *BMC Plant Biol* 27:1299. 10.1186/1471-2229-12-99

8. Dawood T, Rieu I, Arts MW, Derksen EB, Mariani C, Visser EJW (2013) Rapid flooding-induced adventitious root development from preformed primordia in *Solanum dulcamara*. *AoB Plants* 6:plt058doi. 10.1093/aobpla/plt058
9. Dawood T, Yang X, Visser EJ, Te Beek TA, Kensche PR, Cristescu SM et al (2016) A Co-Opted Hormonal Cascade Activates Dormant Adventitious Root Primordia upon Flooding in *Solanum dulcamara*. *Plant Physiol* 170:2351–2364. 10.1104/pp.15.00773
10. Druege U, Franken P, Hajirezaei MR (2016) Plant Hormone Homeostasis, Signaling, and Function during Adventitious Root Formation in Cuttings. *Front Plant Sci* 31:381. 10.3389/fpls.2016.00381
11. Gibbs J, Greenway H, Review (2003) Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Funct Plant Biol* 30(1):1–47. 10.1071/PP98095
12. Haddadi BS, Hassanpour H, Niknam V (2016) Effect of salinity and waterlogging on growth, anatomical and antioxidative responses in *Mentha aquatica* L. *Acta Physiol Plant* 38:119. 10.1007/s11738-016-2137-3
13. Hsu FC, Chou MY, Peng HP, Chou SJ, Shih MC (2011) Insights into hypoxic systemic responses based on analyses of transcriptional regulation in *Arabidopsis*. *PLoS ONE* 6:e28888. 10.1371/journal.pone.0028888
14. Jackson MB, Colmer TD (2005) Response and adaptation by plants to flooding stress. *Ann Bot* 96:501–505. 10.1093/aob/mci205
15. Juntawong P, Sirikhachornkit A, Pimjan R, Sonthirod C, Sangsrakru D, Yoocha T et al (2014) Elucidation of the molecular responses to waterlogging in *Jatropha* roots by transcriptome profiling. *Front Plant Sci* 5:658. 10.3389/fpls.2014.00658
16. Lee TG, Jang CS, Kim JY, Kim DS, Park JH, Kim DY et al (2007) A myb transcription factor (TaMyb1) from wheat roots is expressed during hypoxia: roles in response to the oxygen concentration in root environment and abiotic stresses. *Physiol Plant* 129:375–385. doi.org/10.1111/j.1399-3054.2006.00828.x
17. Licausi F, Ohme-Takagi M, Perata P (2013) APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. *New Phytol* 199:639–649. 10.1111/nph.12291
18. Lorbiecke R, Sauter M (1999) Adventitious root growth and cell-cycle induction in deepwater rice. *Plant Physiol* 119:21–30. 10.1104/pp.119.1.2
19. Loreti E, van Veen H, Perata P (2016) Plant responses to flooding stress. *Curr Opin Plant Biol* 33:64–71. 10.1016/j.pbi.2016.06.005
20. Macgregor DR, Deak KI, Ingram PA, Malamy JE (2008) Root system architecture in *Arabidopsis* grown in culture is regulated by sucrose uptake in the aerial tissues. *Plant Cell* 20(10):2643–2660. 10.1105/tpc.107.055475
21. Mano Y, Omori F (2013) Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zeanicaraguensis*) in maize (*Zea mays* subsp. *mays*). *Ann Bot* 112(6):1125–1139. 10.1093/aob/mct160

22. Mitsuda N, Ohme-Takagi M (2009) Functional analysis of transcription factors in Arabidopsis. *Plant Cell Physiol* 50:1232–1248. 10.1093/pcp/pcp075
23. Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. *BiochimBiophys Acta* 1819:86–96. 10.1016/j.bbagr.2011.08.004
24. Ferner E, Rennenberg H, Kreuzwieser J (2012) Effect of flooding on C metabolism of flood-tolerant (*Quercus robur*) and non-tolerant (*Fagus sylvatica*) tree species. *Tree Physiol* 32(2):135–145. 10.1093/treephys/tps009
25. Fukao T, Xu K, Ronald PC, Bailey-Serres J (2006) A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* 20:21–34. doi:10.1105/tpc.106.043000
26. Gonin M, Bergougnoux V, Nguyen TD, Gantet P, Champion A (2019) What Makes Adventitious Roots? *Plants* (Basel). 22(7):240. 10.3390/plants8070240
27. Kreuzwieser J, Rennenberg H (2014) Molecular and physiological responses of trees to waterlogging stress. *Plant Cell Environ* 37(10):2245–2259. 10.1111/pce.12310
28. Phukan UJ, Mishra S, Timbre K, Luqman S, Shukla RK (2014) *Mentha arvensis* exhibit better adaptive characters in contrast to *Mentha piperita* when subjugated to sustained waterlogging stress. *Protoplasma* 251:603–614. doi: 10.1007/s00709-013-0561-4
29. Phukan UJ, Jeena GS, Tripathi V, Shukla RK (2018) MaRAP2-4, a waterlogging-responsive ERF from *Mentha*, regulates bidirectional sugar transporter AtSWEET10 to modulate stress response in *Arabidopsis*. *Plant Biotechnol J* 16:221–233. 10.1111/pbi.12762
30. Qi X, Li Q, Ma X, Qian C, Wang H, Ren N, Shen C, Huang S, Xu X, Xu Q, Chen X (2019) Waterlogging-induced adventitious root formation in cucumber is regulated by ethylene and auxin through reactive oxygen species signalling. *Plant Cell Environ* 42(5):1458–1470
31. Qi X, Li Q, Shen J, Qian C, Xu X, Xu Q, Chen X (2020) Sugar enhances waterlogging-induced
32. adventitious root formation in cucumber by promoting auxin transport and signalling. *Plant Cell Environ* 43(6):1545–1557. doi: 10.1111/pce.13738
33. Rich SM, Ludwig M, Colmer TD (2012) Aquatic adventitious root development in partially and completely submerged wetland plants *Cotula coronopifolia* and *Meionectes brownii*. *Ann Bot* 110(2):405–414. 10.1093/aob/mcs05
34. Rosa M, Prado C, Podazza G, Interdonato R, Gonzalez JA, Hilal M et al (2009) Soluble sugars—metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal Behav* 4:388–393. 10.4161/psb.4.5.8294
35. Sasidharan R, Voisenek LA (2015) Ethylene-Mediated Acclimations to Flooding Stress. *Plant Physiol* 169:3–12. 10.1104/pp.15.00387
36. Steffens B, Wang J, Sauter M (2006) Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. *Planta* 223:604–612. 10.1007/s00425-005-0111-1

37. Steffens B, Kovalev A, Gorb SN, Sauter M (2012) Emerging roots alter epidermal cell fate through mechanical and reactive oxygen species signalling. *Plant Cell* 24:3296–3306. [10.1105/tpc.112.101790](https://doi.org/10.1105/tpc.112.101790)
38. Steffens B, Rasmussen A (2016) The Physiology of Adventitious Roots. *Plant Physiol* 170(2):603–617. [10.1104/pp.15.01360](https://doi.org/10.1104/pp.15.01360)
39. Takahashi F, Sato-Nara K, Kobayashi K, Suzuki M, Suzuki H (2003) Sugar-induced adventitious roots in *Arabidopsis* seedlings. *J Plant Res* 116(2):83–91. [10.1007/s10265-002-0074-2](https://doi.org/10.1007/s10265-002-0074-2)
40. Teoh EY, Teo CH, Baharum NA, Pua TL, Tan BC (2022) Waterlogging Stress Induces Antioxidant Defense Responses, Aerenchyma Formation and Alters Metabolisms of Banana Plants. *Plants (Basel)* 5(15):2052
41. Vidoz ML, Loreti E, Mensuali A, Alpi A, Perata P (2010) Hormonal interplay during adventitious root formation in flooded tomato plants. *Plant J* 63:551–562. [org/10.1111/j.1365-3113X.2010.04262.x](https://doi.org/10.1111/j.1365-3113X.2010.04262.x)
42. Visser E, Cohen JD, Barendse G, Blom C, Voeselek L (1996) An Ethylene-Mediated Increase in Sensitivity to Auxin Induces Adventitious Root Formation in Flooded *Rumex palustris* Sm. *Plant Physiol* 112(4):1687–1692. [10.1104/pp.112.4.1687](https://doi.org/10.1104/pp.112.4.1687)
43. Voeselek LACJ, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. *New Phytol* 206(1):57–73. [10.1111/nph.13209](https://doi.org/10.1111/nph.13209)
44. Vu J, Yelenosky G (1991) Photosynthetic responses of citrus trees to soil flooding. *Physiol Plant* 81:7–14
45. Wample RL, Reid DM (1978) Control of adventitious root production and hypocotyl hypertrophy of sunflower (*Helianthus annuus*) in response to flooding. *Physiol Plant* 44:351–358. [org/10.1111/j.1399-3054.1978.tb01636.x](https://doi.org/10.1111/j.1399-3054.1978.tb01636.x)
46. Wany A, Kumari A, Gupta KJ (2017) Nitric oxide is essential for the development of aerenchyma in wheat roots under hypoxic stress. *Plant Cell Environ* 40(12):3002–3017. [10.1111/pce.13061](https://doi.org/10.1111/pce.13061)
47. Wu H, Chen H, Zhang Y et al (2019) Effects of 1-aminocyclopropane-1-carboxylate and paclobutrazol on the endogenous hormones of two contrasting rice varieties under submergence stress. *Plant Growth Regul* 87:109–121. [org/10.1007/s10725-018-0457-6](https://doi.org/10.1007/s10725-018-0457-6)
48. Xu X, Chen M, Ji J, Xu Q, Qi X, Chen X (2017) Comparative RNA-seq based transcriptome profiling of waterlogging response in cucumber hypocotyls reveals novel insights into the de novo adventitious root primordia initiation. *BMC Plant Biol* 17:129. [10.1186/s12870-017-1081-8](https://doi.org/10.1186/s12870-017-1081-8)
49. Xu J, Qiao X, Tian Z, Zhang X, Zou X, Cheng Y (2018) Proteomic Analysis of Rapeseed Root Response to Waterlogging Stress. *Plants (Basel)* 7:71. [10.3390/plants7030071](https://doi.org/10.3390/plants7030071)
50. Yamauchi T, Tanaka A, Tsutsumi N, Inukai Y, Nakazono M (2020) A Role for Auxin in Ethylene-Dependent Inducible Aerenchyma Formation in Rice Roots. *Plants (Basel)* 11(5):610. [10.3390/plants9050610](https://doi.org/10.3390/plants9050610)
51. Zhang J, Davies WJ (1987) ABA in Roots and Leaves of Flooded Pea Plants. *J Exp Bot.* 38:649–659. [doi:org/10.1093/jxb/38.4.649](https://doi.org/10.1093/jxb/38.4.649)

52. Zhang Y, Kong X, Dai J, Luo Z, Li Z, Lu H (2017) Global gene expression in cotton (*Gossypium hirsutum* L.) leaves to waterlogging stress. PLoS ONE 12(9):e0185075. 10.1371/journal.pone.0185075
53. Zhang G, Koji T, Abe J, Morita S (2007) Influence of waterlogging on some antioxidative enzymatic activities of two barley genotypes differing in anoxia tolerance. Acta Physiol Plant 29:171–176. 10.1007/s11738-006-0022-1
54. Zhao N, Li C, Yan Y, Cao W, Song A, Wang H et al (2018) Comparative Transcriptome Analysis of Waterlogging-Sensitive and Waterlogging-Tolerant Chrysanthemum morifolium Cultivars under Waterlogging Stress and Reoxygenation Conditions. Int J Mol Sci 19:1455. 10.3390/ijms19051455

Figures

Fig1

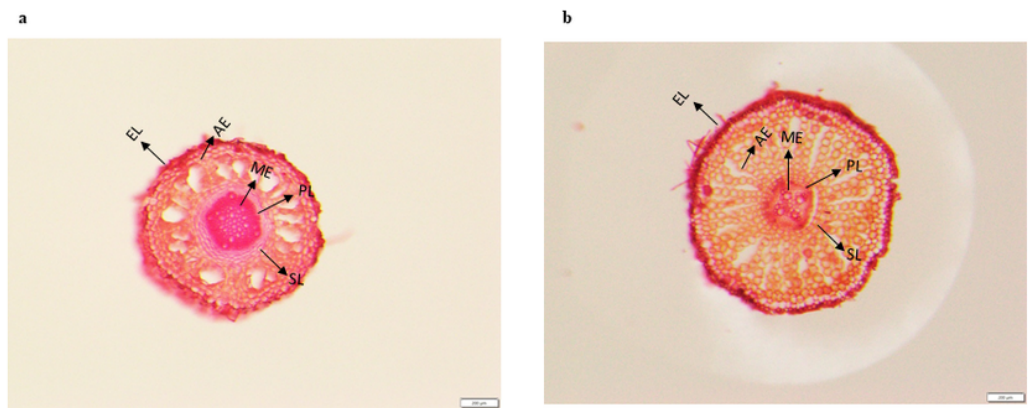


Figure 1

Cross-section analysis of *M. arvensis* plants under waterlogging induced adventitious root and control root **(a)** Control root cross-section examination using light microscopy of *M. arvensis* plant. **(b)** Adventitious roots developed after eight days of waterlogging were utilized for cross-section. Images represented were taken at 2.5X magnification. The scale bars are 200µM.

Fig 2

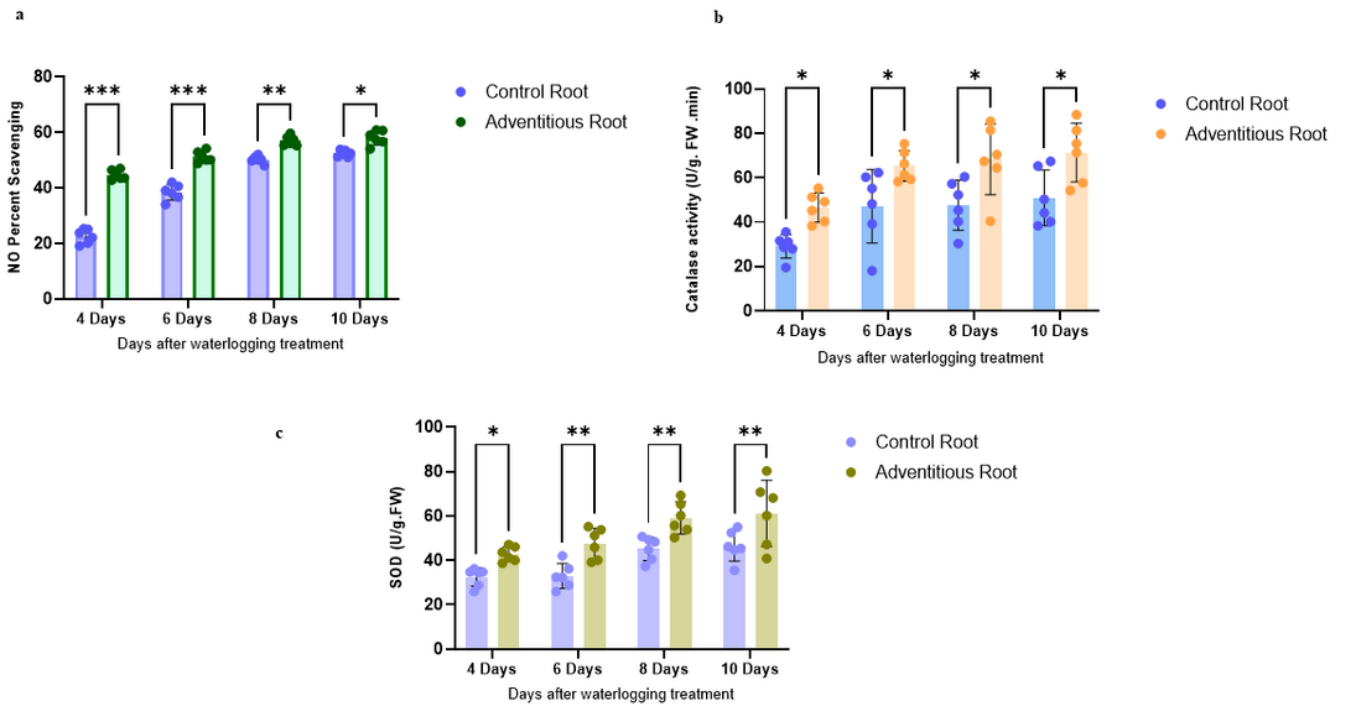


Figure 2

Measurement of antioxidant activity of control root and adventitious root after 4, 6, 8 and 10 days of waterlogging treatment **(a)** Nitric oxide (% Scavenging was calculated by taking absorbance at 546nm) **(b)** Catalase (O.D was measured at 610 nm and expressed in terms of Enzyme unit/g. fresh weight. Min) **(c)** SOD (O.D was taken at 560 nm and expressed as U/g.freshweight. min). Antioxidant activity was enhanced in the waterlogging-induced adventitious root compared to the control root. Error bar indicates $\pm$ SD from two biological replicates, and the data was analyzed statistically. Student's *t-test****P<0.001 **P< 0.01 and * P< 0.05.

Fig 3

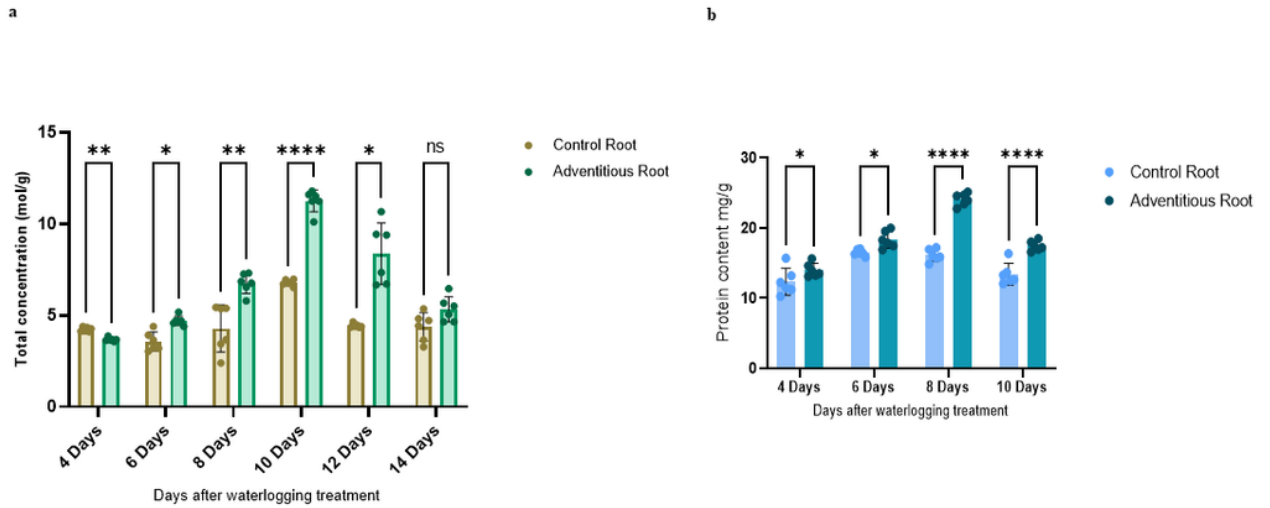


Figure 3

(a) Measurement of total carbohydrate concentration in adventitious root compared to control root expressed in mol/g. **(b)** Total soluble protein content in adventitious root is compared to control root, expressed in terms of mg/g. Adventitious root showed more carbohydrate and protein content than the control root after different days of waterlogging. Error bar indicates $\pm$ SD from two biological replicates, and the data was analyzed statistically. Student's *t-test* **** $P < 0.0001$ *** $P < 0.001$ ** $P < 0.01$ and * $P < 0.05$.

Fig 4

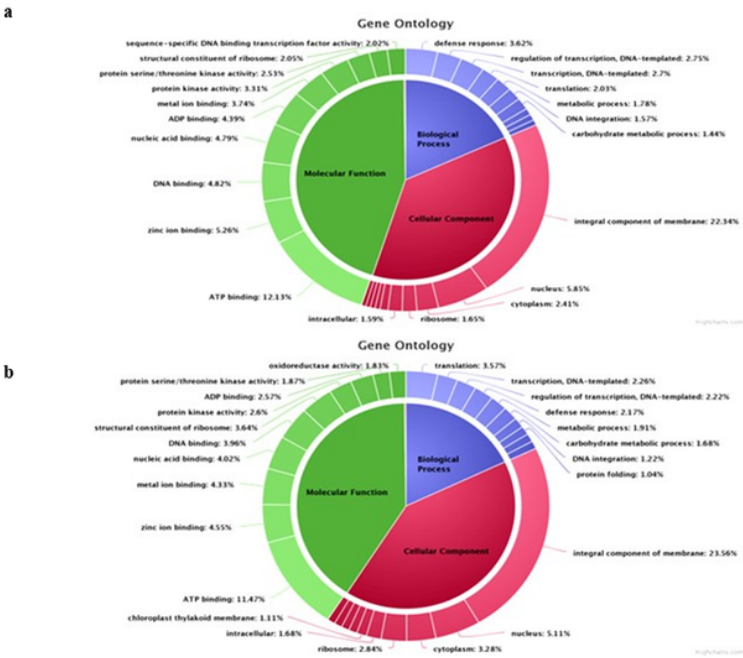


Figure 4

Most representative GO terms based on transcript abundance in each of the three GO domains, i.e., Biological processes, Cellular components, and Molecular function in both **(a)**Control Root and **(b)** Adventitious root. Data represented in the pi-chart is concluded after two independent biological and technical replicates (n = 2).

Fig 5

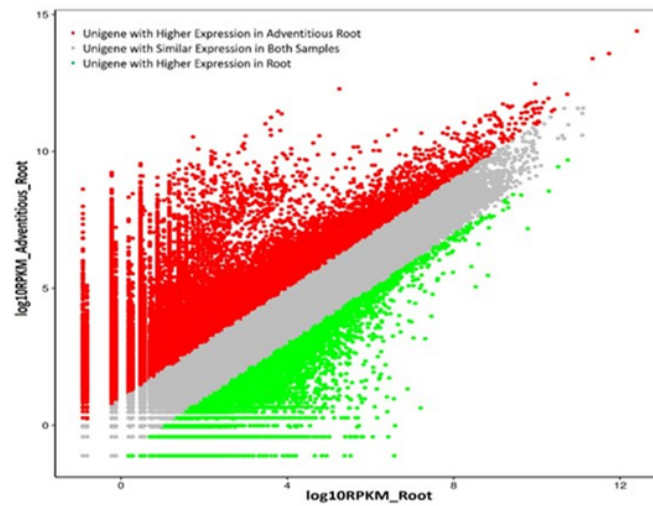


Figure 5

Scattered plot distribution and transcripts expression in Adventitious root (Red dots) and Control roots (Green dots). The middle zone (Grey dots) represents transcripts that were found to be expressed in both control and adventitious roots. Data illustrated in pi-chart is drawn after two independent biological and technical replicates (n = 2).

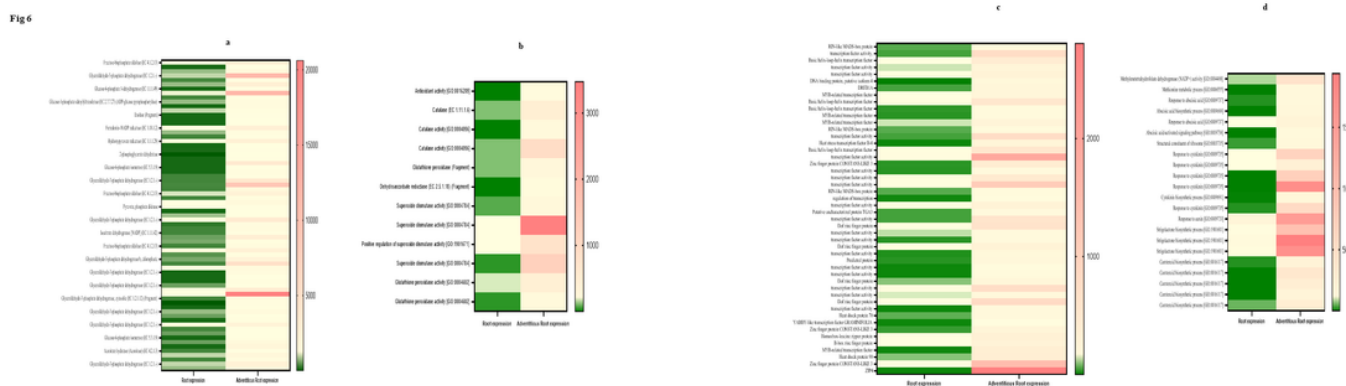


Figure 6

Heat-map of differentially expressed transcripts in adventitious (after waterlogging treatment) and control root selected based on significant *P value* (<0.05) from the transcriptome data of two independent biological and technical replicates (n=2) of *M. arvensis*. Majority of selected transcripts belong to **(a)** Carbohydrate metabolism, **(b)** Antioxidant activity, **(c)** Transcription Factors and **(d)** Hormonal regulation

Relative expression of selected twenty-four upregulated transcripts in adventitious root as compared to control root as well as specifically upregulated in the adventitious root by utilizing qRT-PCR analysis: Most of the transcripts were found to be upregulated after waterlogging treatment validating the transcriptome data. Actin was used as the endogenous reference gene, and the comparative abundance of all the transcripts in the control root and adventitious root was analysed **(a)** Real-Time PCR validation using adventitious roots emerged after 4 days of waterlogging and **(b)** Real-Time PCR validation using adventitious roots emerged after 8 days of waterlogging treatment were analyzed. The data depict three independent biological and experimental replicates. Error bar represents standard deviations. Control was taken as '1'. Student's *t-test* **** P<0.0001 *** P<0.001 ** P< 0.01 and * P< 0.05.

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