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DATA NOTE

RNA-Seq analysis of genes affected by Cyclophilin A/DIAGEOTROPICA (DGT) in tomato root development [version 1; peer review: 2 approved]

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Abstract
Cyclophilin A/DIAGEOTROPICA (DGT) has been linked to auxin-regulated development in tomato and appears to affect multiple developmental pathways. Loss of DGT function results in a pleiotropic phenotype that is strongest in the roots, including shortened roots with no lateral branching. Here, we present an RNA-Seq dataset comparing the gene expression profiles of wildtype ('Ailsa Craig') and *dgt* tissues from three spatially separated developmental stages of the tomato root tip, with three replicates for each tissue and genotype. We also identify differentially expressed genes, provide an initial comparison of genes affected in each genotype and tissue, and provide the pipeline used to analyze the data. Further analysis of this dataset can be used to gain insight into the effects of DGT on various root developmental pathways in tomato.

Keywords
Tomato, Root, Development, Diageotropica, DGT, RNA-Seq, Gene expression

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Author roles: Ivanchenko MG: Conceptualization, Funding Acquisition, Investigation, Writing – Original Draft Preparation; **Ozguc OR**: Formal Analysis, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Bollmann SR**: Formal Analysis, Writing – Review & Editing; **Fraser VN**: Formal Analysis, Writing – Review & Editing; **Megraw M**: Funding Acquisition, Supervision, Writing – Review & Editing

Introduction

The tomato (*Solanum lycopersicum*) cyclophilin DIAGEO-TROPICA (DGT) has been linked to auxin-regulated development through identification of the gene affected by the *diageotropica* (*dgt*) mutation[1,2]. Tomato *dgt* mutants are auxin-resistant and display a pleiotropic phenotype that includes slow gravitropic response, lack of lateral root initiation, altered vascular development, reduced ethylene production in response to auxin, reduced apical dominance, impaired shoot and root growth, reduced fertility, and impaired fruit growth[3–11]. DGT likely interacts with auxin transport and signaling in a complex manner. For instance, DGT has been shown to negatively regulate auxin efflux via PIN-FORMED (PIN) transporters by altering subcellular localization of PINs and expression of some *PIN* genes[9]. *DGT*, in turn, is downregulated by auxin at the root tip[8], suggesting functional feedback between DGT, auxin, and PINs. Through targeted gene expression quantification with RT-PCR and northern blots, DGT has been demonstrated to affect expression of a number of other auxin-related genes in addition to *PIN* genes. The *dgt* mutation reduces auxin-induced expression of genes encoding certain 1-aminocyclopropane-1-carboxylic acid synthases (ACCSs; key ethylene biosynthesis regulatory enzymes), SMALL AUXIN UPREGULATED RNA (SAUR) genes, and several members of the auxin-regulated *Aux/IAA* gene family in a tissue- and developmental stage-specific manner[4,6,10–14]. However, full transcriptomic profiling of tomato *dgt* mutants in different developmental zones has not been performed. Given the complex role of DGT in auxin pathways, an exploration into the widespread effects of DGT on the transcriptome may provide valuable insights into its potentially extensive and multifaceted role in development.

In this study, we perform a global analysis of gene expression in *dgt* roots that compares the root meristem, elongation zone, and differentiation zone in wildtype ('Ailsa Craig') and *dgt* tomato plants. The root tip provides an excellent system for studying development-related plant gene expression because cell division, elongation, and maturation are not only temporally but also spatially separated in this growth region; this allows for anatomical dissection and analysis of specific developmental zones, such as the meristem, elongation zone, and differentiation zone. The root tip is also most appropriate for this study because the *dgt* phenotype is the strongest in the root tip and has been characterized morphologically in the root tip[7,8]. We have previously performed histological analyses of tomato root tips including the meristem, elongation zone, and maturation zone, and demonstrated a decrease in length and number of cells of the *dgt* meristem and elongation zone[8], whereas the initiation of lateral root primordia was abolished in the *dgt* root maturation zone[7–9]. Here, we present an RNA-Seq dataset containing raw reads and abundance estimates for three replicates in each zone and genotype, the pipeline used for analysis, and an initial exploration of expressed and differentially expressed genes in each developmental region that can be used to guide future investigations.

Materials and methods

Plant material and growth conditions

Three biological replicates for each tissue and genotype were performed. Seven-day old tomato (*Solanum lycopersicum*) wildtype (WT) and *dgt^1-1^* plants in the 'Ailsa Craig' background[7] were used. Seeds were sterilized in 20% commercial bleach for 30 min and rinsed four times for 10 min with sterile water. Sterilized seeds were vernalized at 4°C for two days to ensure even germination and then planted on media containing 0.2× Murashige and Skoog basal medium with vitamins (PhytoTechnology), 1% sucrose, 10 mM MES buffer pH 5.7, and 0.8% agar. Seeds were germinated in Magenta boxes (16 seeds per box) in a growth chamber at 21°C under long day (16h light, 8h dark) conditions and light intensity as in Ivanchenko *et al.* (2013). Root samples were dissected under DIC optics at 4x objective as in Ivanchenko *et al.* (2006). On average, 50–100 root portions were collected per biological replicate. The meristem was dissected between the root tip and the root portion where tissue becomes more transparent. The elongation zone was collected from the proximal meristem border and the first hair bulge, and approximately 1 cm portions were collected above the first hair bulge for the differentiation zone.

RNA isolation and RNA-Seq

Tissue samples were collected in Plant RNA Reagent (Life Technologies) on ice, and total RNA was prepared using the RNeasy Mini kit (Qiagen) according to manufacturer's recommendations. The RNA pellets were dried in 1.7 mL centrifuge tubes and solubilized in 178 μL 1X RNA Secure Reagent (Ambion) preheated at 65°C, and incubated at 65°C for 10 min, mixing by pipetting a few times. Then 20 μL 10X DNase I buffer and 2 μL RNase-free DNase I (Ambion) were added to each tube, and tubes incubated for 10 min at 37°C. 700 μL RLT buffer was added (to which 7 μL 2-Mercaptoethanol was freshly added) to each sample, which were then mixed by vortexing. 500 μL ethanol was then added and samples were mixed again by vortexing. Each sample (2 X 700 μl) was applied to an RNeasy Mini spin column from the RNeasy kit and RNA cleanup performed following the manufacturer's instructions. Each sample was eluted with 30 μL nuclease-free ultrapure H_2O, and the RNA concentrations were measure using a NanoDrop 1000 spectrophotometer (Thermo Fisher).

Illumina sequencing of RNA

RNA-Seq library preparation and sequencing were performed at the Oregon State University Center for Genome Research and Biocomputing. Libraries were prepared using the TruSeq RNA Library Prep Kit v2 (Illumina) and sequenced as single-end 51 bp reads on the Illumina HiSeq 2000 using a total of two lanes.

Gene alignment and expression level analysis

Sequencing produced 5-8 FASTQ files per replicate, which were merged into a single FASTQ file per replicate. Reference transcripts were extracted and preprocessed from the NCBI *Heinz 1706* genome sequence[15] using RSEM's[16] (RNA-Seq by Expectation Maximization, version 1.3.1) rsem-prepare-reference function. Using RSEM, raw sequence reads were then aligned to the reference transcript sequences and estimated transcripts per million (TPM) values were calculated using default parameters. Resulting gene-level estimates from biological replicates were merged into a single input matrix and EBSeq[17] (version 1.26.0) was then used to test for differential expression between *dgt* and WT for each root-tip zone. Raw RNA-Seq reads, abundance estimates, and differential expression analysis are publicly available

in the Sequence Read Archive (SRA) and Gene Expression Omnibus (GEO), see *Data availability*.

Data visualization

As a preliminary analysis to guide future investigations, a basic comparison of presence/absence calls and differential gene expression is presented. Expression analysis was performed using TPM and differential expression analysis was performed using the posterior probability that the gene is differentially expressed (PPDE) and posterior fold change (postFC). Genes with an average of TPM > 2 across biological replicates were compared between zones and genotypes (Figure 1; data summarized in Data File 1[18]). Following EBSeq analysis, genes were filtered for PPDE = 1 to identify those which were differentially expressed.
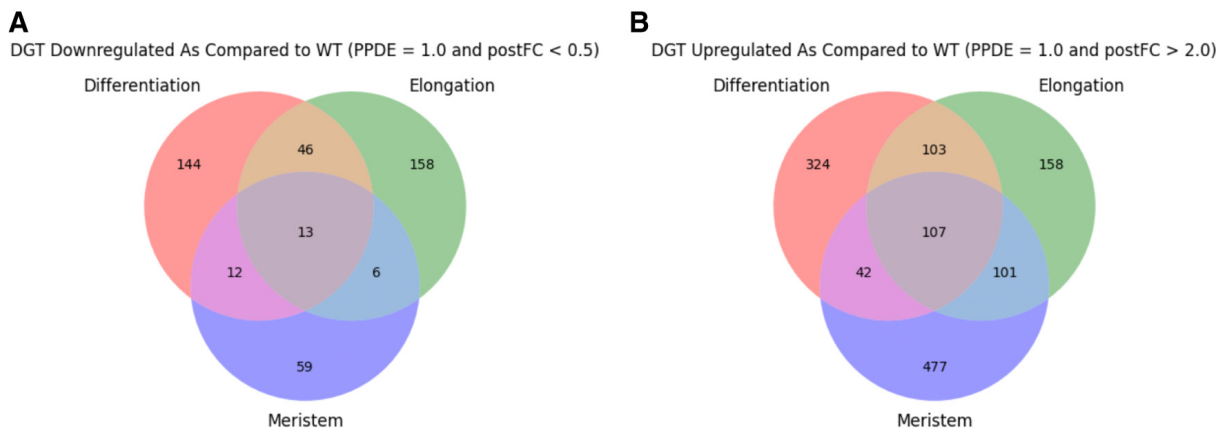
Of the differentially expressed genes, those with postFC (*dgt* over WT) > 2 were considered upregulated in *dgt* and those with postFC < 0.5 were considered downregulated in *dgt*. Upregulated and downregulated genes were compared between zones and genotypes (Figure 2; data summarized in Data File 2[19]). Principal component analysis (PCA) of the data was performed using TPM values of each gene and was calculated using the scikit-learn[20] PCA function with default parameters (Figure 3).

Conclusion

It is clear from the outcomes in Figure 1 and Figure 2 that reduced function of DGT has sweeping effects on the transcriptome in all three developmental zones examined in this study, supporting the concept that DGT very likely plays important and potentially
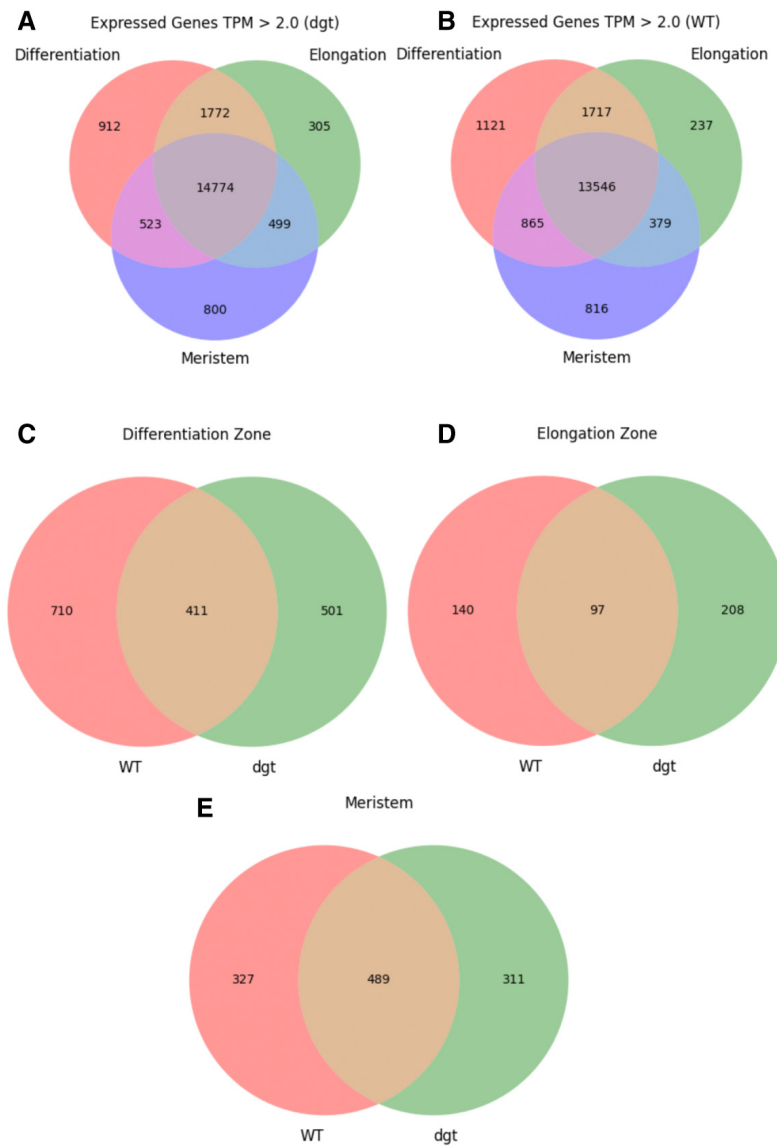
Figure 1. Presence/absence calls. (**A**) Number of genes expressed in each zone of *dgt* roots, transcripts per million (TPM) > 2.0. (**B**) Number of genes expressed in each zone of WT roots, TPM > 2.0. (**C**) Number of genes expressed exclusively in the differentiation zone with TPM > 2.0, compared between WT and *dgt*. (**D**) Number of genes expressed exclusively in the elongation zone with TPM > 2.0, compared between WT and *dgt*. (**E**) Number of genes expressed exclusively in the meristem with TPM > 2.0, compared between WT and *dgt*.

Figure 2. Differentially expressed genes. (**A**) Differentially expressed genes downregulated in each zone with postFC < 0.5, posterior probability that the gene is differentially expressed (PPDE) = 1 in *dgt* vs WT. (**B**) Differentially expressed genes upregulated in each zone with postFC > 2, PPDE = 1 in *dgt* vs. WT.
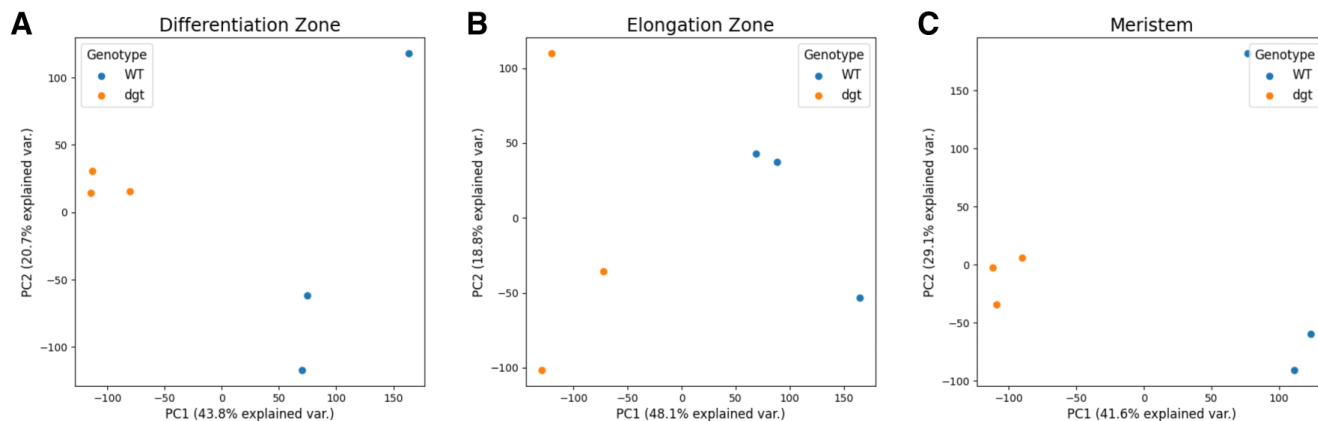
Figure 3. Principle component analysis (PCA). (**A**) PCA of sequenced samples in differentiation zone (**B**) PCA of sequenced samples in elongation zone (**C**) PCA of sequenced samples in meristem.

complex roles in multiple developmental pathways. Additionally, PCA using TPM values for each sample demonstrated that while there was some variance within the replicate pools, replicates from different genotypes were distinctly separate from each other (Figure 3). Further functional genomics studies are needed to narrow down the most likely direct interactions with DGT, leading to the identification of specific functional roles. We hope that this dataset will be of value to the community in future studies in this area.

Data availability

Source data
Heinz 1706 genome available from Assembly, Accession number GCF_000188115.4: https://www.ncbi.nlm.nih.gov/assembly/GCF_000188115.4/

Underlying data
Raw RNA-Seq reads and expression estimates provided by RSEM on Gene Expression Omnibus, Accession number GSE156398: https://identifiers.org/geo:GSE156398

Figshare: Data File 1: Genes referenced in figure 1. https://doi.org/10.6084/m9.figshare.12891773.v1[18].

Figshare: Data File 2: Genes referenced in figure 2. https://doi.org/10.6084/m9.figshare.12891941.v1[19].

Software availability
Source code available from: https://github.com/ozguco/tomato_dgt_RNASeq

Archived source code at time of publication: https://doi.org/10.5281/zenodo.4029399[21]

License: GNU General Public License v3.0

References

1. Oh K, Hardeman K, Ivanchenko MG, *et al.*: **Fine mapping in tomato using microsynteny with the *Arabidopsis* genome: the *Diageotropica (Dgt)* locus.** *Genome Biol.* 2002; **3**(9): research0049.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

2. Oh K, Ivanchenko MG, White TJ, *et al.*: **The *diageotropica* gene of tomato encodes a cyclophilin: a novel player in auxin signaling.** *Planta.* 2006; **224**(1): 133–144.
   PubMed Abstract | **Publisher Full Text**

3. Zobel RW: **Some Physiological Characteristics of the Ethylene-requiring Tomato Mutant Diageotropica.** *Plant Physiol.* 1973; **52**(4): 385–389.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

4. Mito N, Bennett AB: **The diageotropica Mutation and Synthetic Auxins Differentially Affect the Expression of Auxin-Regulated Genes in Tomato.** *Plant Physiol.* 1995; **109**(1): 293–297.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

5. Rice MS, Lomax TL: **The auxin-resistant *diageotropica* mutant of tomato responds to gravity via an auxin-mediated pathway.** *Planta.* 2000; **210**(6): 906–913.
   PubMed Abstract | **Publisher Full Text**

6. Balbi V, Lomax TL: **Regulation of Early Tomato Fruit Development by the *Diageotropica* Gene.** *Plant Physiol.* 2003; **131**(1): 186–197.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

7. Ivanchenko MG, Coffeen WC, Lomax TL, *et al.*: **Mutations in the *Diageotropica (Dgt)* gene uncouple patterned cell division during lateral root initiation from proliferative cell division in the pericycle.** *Plant J.* 2006; **46**(3): 436–447.
   PubMed Abstract | **Publisher Full Text**

8. Ivanchenko MG, den Os D, Monshausen GB, *et al.*: **Auxin increases the hydrogen peroxide (H2O2) concentration in tomato (*Solanum lycopersicum*) root tips while inhibiting root growth.** *Ann Bot.* 2013; **112**(6): 1107–1116.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Ivanchenko MG, Zhu J, Wang B, *et al.*: **The cyclophilin A DIAGEOTROPICA gene affects auxin transport in both root and shoot to control lateral root formation.** *Development.* 2015; **142**(4): 712–721.
   PubMed Abstract | **Publisher Full Text**

10. Bradford KJ, Yang SF: **Stress-induced Ethylene Production in the Ethylene-requiring Tomato Mutant Diageotropica.** *Plant Physiol.* 1980; **65**(2): 327–330.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

11. Kelly MO, Bradford KJ: **Insensitivity of the *diageotropica* tomato mutant to auxin.** *Plant Physiol.* 1986; **82**(3): 713–717.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

12. Nebenführ A, White TJ, Lomax TL: **The diageotropica mutation alters auxin induction of a subset of the Aux/IAA gene family in tomato.** *Plant Mol Biol.* 2000; **44**(1): 73–84.
    PubMed Abstract | **Publisher Full Text**

13. Coenen C, Christian M, Lüthen H, *et al.*: **Cytokinin inhibits a subset of diageotropica-dependent primary auxin responses in tomato.** *Plant Physiol.* 2003; **131**(4): 1692–1704.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Vidoz ML, Loreti E, Mensuali A, *et al.*: **Hormonal interplay during adventitious root formation in flooded tomato plants.** *Plant J.* 2010; **63**(4): 551–562.
    PubMed Abstract | **Publisher Full Text**

15. Tomato Genome Consortium: **The tomato genome sequence provides insights into fleshy fruit evolution.** *Nature.* 2012; **485**(7400): 635–641.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

16. Li B, Dewey CN: **RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome.** *BMC Bioinformatics.* 2011; **12**: 323.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

17. Leng N, Kendziorski C: **EBSeq: An R package for gene and isoform differential expression analysis of RNA-seq data**. R package version 1.28.0. 2020.
    Reference Source

18. Ozguc O: **Data File 1: Genes referenced in figure 1.** *figshare.* Dataset. 2020.
    http://www.doi.org/10.6084/m9.figshare.12891773.v1

19. Ozguc O: **Data File 2: Genes referenced in figure 2.** *figshare.* Dataset. 2020.
    http://www.doi.org/10.6084/m9.figshare.12891941.v1

20. Pedregosa F, Varoquaux G, Gramfort A, *et al.*: **Scikit-learn: Machine Learning in Python.** *J Mach Learn Res.* 2011; **12**: 2825–2830.
    Reference Source

21. ozguco:**ozguco/tomato_dgt_RNASeq: First release (Version v1.0.0).** *Zenodo.* 2020.
    http://www.doi.org/10.5281/zenodo.4029399

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Version 1

Reviewer Report 16 October 2020

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✓

Aurélien Bailly (iD)

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Ivanchenko and colleagues describe here a transcriptomics data set profiling gene expression in root tips of the tomato mutant *dgt* and matching wildtype. As for several plant immunophilins, loss of function of DIAGETROPICA triggers dramatic structural and behavioural phenotypes in tomato. The link between immunophilins and hormonal control has been extensively recognised; however, the detailed molecular mechanisms behind them remain elusive.

The interest in this RNAseq dataset lies within the dissection of the root into the three standard spatio-temporal zones of development. Gaining insight into the effect of the *dgt* mutation in the transcriptomes of the meristematic, elongation and mature zones of the root would potentially lead to the isolation of long-sought components of root growth and development machineries.

The work presented here is technically sound and used state of the art transcripts-reading methods. Data deposition was correctly performed and gene lists produced after alignments and differential expression analysis through regular scripts are clear and readily exploitable. Although the level of variation between replicates, as mentioned by authors, do vary in the different root zones samples, I trust that they separate enough in PCA to yield interesting outcomes. In addition, the relatively reduced number of differentially expressed genes filtered in this analysis will allow for a rapid identification of proteins associated with the dgt phenotype, perhaps outside of the classical pool of auxin-related genes.

I would express only two regrets: 1) the DGT protein was shown to hold a conserved auxin signalling function down to moss, but it is not mentioned here; and 2) other immunophilins present overlapping roles with DGT, a paradigm worth mentioning somewhere in the introduction.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 12 October 2020

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The article by Maria Ivanchenko *et al*. "RNA-Seq analysis of genes affected by Cyclophilin A/DIAGEOTROPICA (DGT) in tomato root development" presents the results of RNAseq analysis of the roots of a tomato mutant affected in Cyclophilin A/DIAGEOTROPICA (DGT) vs wild type. The authors compare three developmental zones in the root tip of mutant and wild type seedlings and identify differentially expressed genes. Considering the pleiotropic effect of the mutation and its relation to auxin signaling and transport, this work is potentially important to understand abnormalities in the root development in the *dgt* mutant and the role of *DGT* in root development. Considering that the formation of lateral root primordia is impaired in *dgt* mutants, this analysis can also be useful in the identification of candidate genes involved in lateral root initiation.

All the raw data are publicly available, the same as the scripts used for the RNA-seq analysis. A minor suggestion: the authors should explicitly state whether any pre-processing steps were performed on the raw sequencing reads, such as removal of low-quality bases, adapter filtering, or trimming. Regarding the PCA analysis, one of the WT replicates does not group closely with the other WT samples. It could be useful if the authors provide some insight on why this occurs and discuss the effect of this on TPM variation among replicates.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: JD: Plant development, root development, root growth and branching, lateral root development. GR-A: Transcriptomics and evolutionary biology.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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