THE ROLE OF TRANSCRIPTION FACTOR NGALS IN THE DEVELOPMENT OF *ARABIDOPSIS* TAPROOT

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Abstract. To explore the NGAL1, NGAL2 and NGAL3 transcription factors of the RAV subfamily of the NGATHA family in *Arabidopsis*, and analyze the function of N gal - tri in *Arabidopsis* root development, from NGAL ngal1-2 ngal2-1 ngal3-1 genes. The loss-of - function triplet mutant Ngal-tri starts with an elongated taproot and develops earlier in the central cortex than the wide type. To investigate the function of NGAL1 in *Arabidopsis* root development, the wild-type *Arabidopsis* Columbia ecotype seeds were selected to construct a phylogenetic tree and analyze motifs to predict the gene function of the transcription factor NGAL family. Plants overexpressing Ngal1-p35s were significantly shorter than the wild type taproot, while the root length of the Ngal1 function loss mutant Ngal1-2 had no significant change compared with the wide type tap root, and the function loss triple mutant Ngal-Tri was significantly longer than the wide type. Analyzing the result, it may be due to the redundancy of Ngal1, Ngal2, and Ngal3 functions caused by the binary vector pCambia1300-pNgal1. NGAL1-GFP was transferred into N gal -Tri, resulting in a longer root system than wide type native roots. The transgenic plant Ngal-Tri p NGAL1:NGAL1-GFP obtained by transferring the binary vector p Cambia1300-p NGAL1:NGAL1-GFP into Ngal-Tri can better restore the abnormal root phenotype of the Ngal-Tri triple loss-of-function mutant, indicating the function of the Ngal family in root development. The design of Bi FC experiments revealed that Ngal1 physically interacts with TMO5 and LHW, respectively. In order to search for the genetic link between the Ngal family and TMO5, the study hybridized the functionally lost quadruple mutant TMO5 Ngal1 Tri. The results showed that under the Ngal Tri background, the loss of TMO5 function weakened Ngal to some extent, resulting in the root elongation and thickening phenotype of the triple function loss mutant. Based on the above research, it can be seen that NGALs transcription factors play a certain role in *Arabidopsis* root development, and this study will also have important reference value for plant root development and plant regulation research.
Keywords: *hybridized, NGAL, Ngal1-p35s, triple mutant, gene expression*

Introduction

The root system is an organ for plants to absorb water and nutrients from the external environment, and it is also a sequestration organ (Yan et al., 2021; Nasiri et al., 2023). The growth of plant roots shows strong plasticity. When the water and nutrients in the soil are unevenly distributed, plants adapt to environmental changes by changing the growth direction of roots (Olas et al., 2021; Khashayarfard et al., 2023). The model plant *Arabidopsis* has the characteristics and advantages of model organisms, such as being short, having a rapid reproduction cycle, large population of offspring and complex genetic research tools (Vaseva et al., 2021). With the development of genomics and gene sequencing technology, the maturity of *Arabidopsis* genome sequencing technology and the establishment of mutant embryo banks have made it easier for scientists to study the function of *Arabidopsis* genes through genetics (Busoms et al., 2021). In recent decades, the study of *Arabidopsis* in leaf and flower development, light and hormone signaling has been an important starting point for the study of other plants (such as cereals) (Afitlhile et al., 2021).

Arabidopsis thaliana is a flowering plant distributed in Asia, Europe and North Africa. The main root develops from the radicle, and its structure is simple (*Fig. 1*),

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which can be divided into meristematic zone, elongation zone and mature zone from the vertical axis; the structure is radial from the horizontal axis, and can be subdivided into single-layered epidermis, cortex, endothelial layer and central column (Maiber et al., 2022). The root cap is located in the outermost layer of the meristem, and its composition includes the lateral root cap and the central root cap. The main function of the root cap is to protect the root tip (Mahana et al., 2022). The cells in the meristematic zone are relatively uniform in size and arranged neatly. At the same time, they can continuously divide and generate new cells. Most of the meristem cells will differentiate into elongate zone cells (Amirzakaria et al., 2022). In the apical tissue of the meristematic zone, quiescent cells do not divide, and stem cells grow around the quiescent center to form a stem cell niche. There is a transition zone between the meristematic zone and the elongation zone. The cells in the elongation zone expand and grow, allowing the root tip of *Arabidopsis* to grow towards the soil; the cells in the mature zone can differentiate into root hairs to obtain water and mineral nutrients (Dai et al., 2022; Nazish et al., 2020).

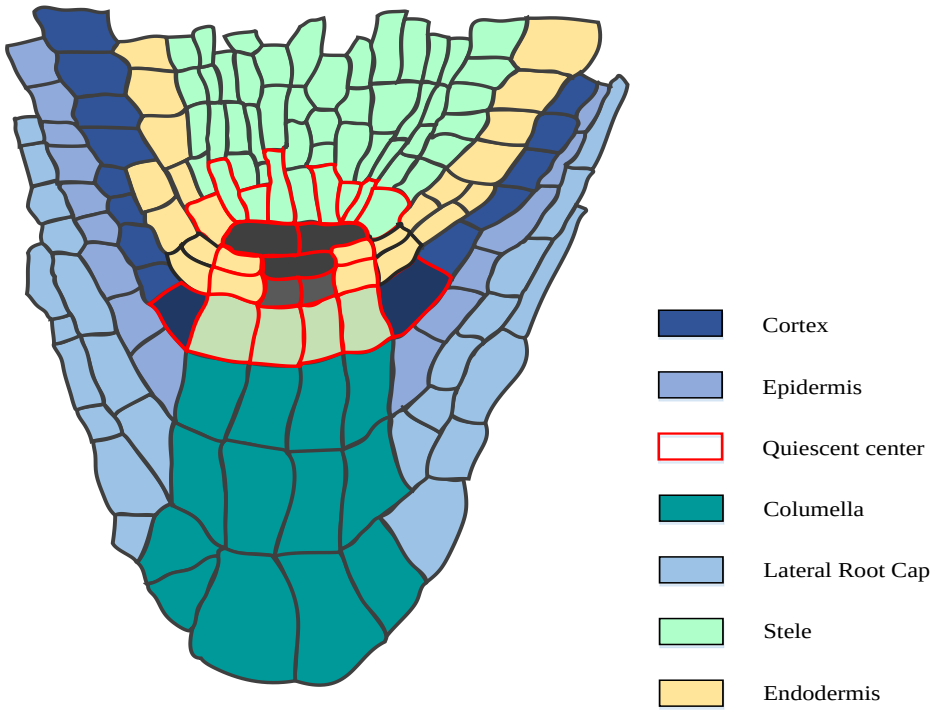

Figure 1. *Arabidopsis main root structure*

The main root of *Arabidopsis thaliana* is divided into vascular tissue, pericycle, inner cortex, cortex and epidermis from inside to outside. The vascular tissue and pericycle are collectively called stele (Alvarez-Rodriguez et al., 2022; Su et al., 2022). There is microtubule tissue in the pericycle, and the microtubule tissue is composed of xylem, phloem, and procambium cells (Tang et al., 2021; Ruchika and Tsukahara et al., 2021; Arain et al., 2021). In a cross-section of a mature *Arabidopsis* tap root, the stele has a centrosymmetric structure, with metaxylem at the center, proxylem at the poles, and two phloems arranged vertically on both sides of the xylem axis (Erp et al., 2021). The cortex and endothelium, known as primitive cells, differentiate through the division of cortical/endothelial subcells, which divide vertically to form daughter cells, which in

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turn divide vertically to form endothelial and cortical progenitors. At the same time, the development of the epidermis also starts from the initial cells around the epidermis (Park et al., 2021; Serafini-Fracassini et al., 2021).

Arabidopsis root apical meristem (RAM) begins to form during embryonic development (Carriquí et al., 2021). The meristem contains some stem cells centered on quiescent centers, their mitotic activity is reduced, and they initiate root development by forming progenitor cells, which are combined with various hormonal signals during development (Yu et al., 2021). The list of genetic material related to *Arabidopsis* thaliana research is shown in *Table 1*.

Table 1. *List of genetic material related to Arabidopsis thaliana research*

References	Genetic material
Alvarez-Rodriguez et al., 2022; Su et al., 2022	*Arabidopsis* vascular tissue and pericycle are collectively referred to as
Tang et al., 2021	Microtubule organization
Erp et al., 2021	Transverse woody structure of mature *Arabidopsis* thaliana main roots
Park et al., 2021	Cortical and endothelial cells
Carriquí et al., 2021	*Arabidopsis* root meristem
Yu et al., 2021	*Arabidopsis* meristem

According to the above research, *Arabidopsis* has biological characteristics and related advantages such as short stature, fast generation cycle, large offspring population, and mature genetic research methods. Especially with the continuous completion and maturity of modern genomics and gene sequencing technology, more and more people have conducted research on *Arabidopsis* and established related plant databases. Despite the lack of in-depth research on *Arabidopsis*, which mainly focuses on the study of plant growth characteristics. Therefore, the study will investigate the role of NGAL transcription factors in the development of *Arabidopsis* main roots, and the research on plant NGAL transcription factors will also be beneficial for people to have a clearer understanding of NGALs, members of the RAV subfamily of *Arabidopsis* B3 transcription factors. This will provide relevant research references for plant growth, development, and regulation processes, and further enrich the regulatory network of *Arabidopsis* leaf edge and main root development.

Materials and methods

Experimental materials

In order to analyze the function and expression patterns of NGALs in *Arabidopsis* thaliana, *Arabidopsis* Col-0 seeds will be selected as experimental materials and stored in the laboratory. Seeds are propagated through hybridization. Among them, Ngal1 Tri pNgal1 GFP is an overexpressed transgenic line with stable heritability obtained in the early stage of the experiment; Ngal1 Tri was mainly obtained by hybridizing ngal-b1 and ngal-b2 in the laboratory; And obtained ngal-b1 (CS68622) and ngal-b2 (SALK200235C) from *Arabidopsis* Biological Resource Center.

Experimental instruments

(1) Instruments: BIO-RAD C1000 PCR181148 gene amplification instrument, Thermo F3 pipetting gun, Eppendorf 5804 desktop high-speed centrifuge, SonyDSC-

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RX10M4 black card digital camera, MSC-100A heating and cooling dry thermostat, DK-98- IIA double-column eight-hole-electric constant temperature water bath, water-proof incubator electric incubator GHP-9050, Leica-M205FCA stereo fluorescence microscope, Thermo Fisher (thermoelectric) thermo Pico17R desktop micro high-speed centrifuge, BIO-RAD Automatic gel imaging system GelDocXR+, Thermo Scientific Precision 818 large-capacity plant incubator, etc.

(2) Olympus IX73 inverted fluorescence microscope, Olympus spinning disk confocal super-resolution microscope IXplore SpinSR.

(3) Enzymes: EasyKOD Plus DNA polymerase (purchased from Takara), T4 DNA Ligase phage DNA ligase (purchased from Thermo), calf intestinal alkaline phosphatase 30 U/μL (Code No. 2250A) (purchased from Takara), endoribonuclease Ribonuclease A (purchased from Takara).

Experimental reagents and configuration methods

(1) Solution: use 25 mL 84 disinfectant, 250 μL 20% TritonX-100, double distilled water to make 50 mL seed rinse solution. Antibiotic solutions are prepared from powdered solutions at the following concentrations. 100 mg/mL ampicillin, 50 mg/mL kanamycin, 25 mg/mL rifamycin, 50 mg/mL gentamicin.

(2) Kits: Thermo Fisher DNA Agarose Gel Kit (spin-column type), Thermo Fisher Scientific Plasmid Mini-Extraction Kit (spin-column type), Tenagen Express Plant Genome Extraction Kit.

(3) Medium: LB medium, MS medium, 1/2 MS medium, see Appendix H for specific configuration.

(4) Thermo Scientific RNase-free reverse transcription kit, Novozymes SYBR® Green I, Tiangen Taq 2× Master Mix.

Research methods

Phylogenetic tree construction and motif analysis

This section is mainly aimed at studying the functional prediction and expression patterns of NGALs, and completing relevant analysis by constructing a phylogenetic tree. Specific process: (1) Obtain the amino acid sequences of *Arabidopsis* RAV members and other closely related proteins from the TAIR database, save the files, and upload the amino acid sequence data. Multiple sequence comparisons were performed on the above data using software, and the largest parse tree was constructed for the comparison results. Finally, analyze the obtained phylogenetic tree and its corresponding protein motif pattern. The predicted results of the promoter region sequence of the transcription factor NGALs gene were obtained from the TAIR database. MEM (Multi Experiments Matrix) was used to predict the target genes of NGAL1, NGAL2 and NGAL3 to find the signaling pathways that may be involved in NGALs.

(2) Obtain the gene sequence of NGALs in the database. Determine the promoter of the transcription factor NGALs, and select the appropriate restriction site to construct the binary vector.

Tissue-specific expression of NGALs transcription factors

This process mainly analyzes the tissue-specific expression of NGALs: (1) Linearize the deoxyribonucleic acid fragments and empty plasmids obtained by

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endonuclease recovery. The specific operation is to place the deoxyribonucleic acid fragments and empty plasmids in a constant temperature water bath at 98.6 ℉ for 60 min. After 60 min CIAP was added to remove the 5’ phosphate group. Place the carrier again in a constant temperature water bath at 98.6 °F for 60 min to collect the digested product. The composition of the specific enzyme digestion reaction solution is shown in *Table 2*.

Table 2. *Enzyme digestion reaction system table*

Reaction system	Total system (30 μL)
DNA	≦ 1 μg
10X QuickCut Green Buffer	5 μL
Restriction Endonuclease	1 μL
Double Distilled Water	Up to 50 μL

(2) Next, connect the fragment with the carrier: mix 1 μ l of the digestion product with 1 μ l of the carrier and 9 μ l of the loading buffer, place it in a constant temperature water bath at 86 ° F for 5 min, conduct agarose gel electrophoresis, and recover the product. According to the amount of recovered product, 0.5 μ l of T4 Ligase is added to every 10 μ l of the total system, mixed well, and placed in a connecting instrument at 71.6 ℉ for 30 min for the connecting reaction.

(3) Then proceed with the conversion of the connected product: Take out 100 μL of competent cells prepared by the frozen TSS method, and put them on ice for thawing. Add the thawed TSS competent cells to the ligation product obtained in step (4) Mix well, place in a constant temperature water bath at 32 ℉ for 30 min, then place in a constant temperature water bath at 107.6 ℉ for 90 s, and add 1 ml of Add Luria-Bertani medium into the reaction tube and incubate for 1 h in a constant temperature shaker at 98.6℉ and 220 r/min, that is, the bacterial solution is applied to the resistant plate and incubated upside down for 8 h.

(4) Identification of bacterial colonies: The monoclonal strains were collected in a test tube containing 1 mL of Luria-Bertani liquid, cultured for 8 h, plasmid DNA was extracted by alkaline lysis, and colonies were identified by enzyme digestion.

(5) Extract plasmids and sequence them: The specific method is to pick the monoclonal colonies of the streaked medium after colony identification, add antibiotics to 30 mL of Luria-Bertani liquid, and place them in a constant temperature shaker at 98.6 ℉ and 220 r/min. Incubate for 18 h. Consume 2 μL of plasmid and primers for sequencing according to one reaction.

(6) Obtaining transgenic plants through inflorescence infection method: T1 generation (mixed harvest) transgenic seeds and T2 generation seeds were obtained by inflorescence infection method.

(7) P NGAL1: Screening of GUS transgenic plants: The T1 generation transgenic seeds were cultured in a dark, 39.2 °F plant incubator, and the seeds were sprayed with Basta herbicide after germination, and positive seedlings with herbicide resistance were obtained.

(8) Screening of transgenic plants with pNGAL1: GFP, pNGAL2: GFP, pNGAL3: GFP: Sow T1 generation seeds on MS solid medium containing antibiotics, culture for 10 days, select positive seedlings containing fluorescent signals, move them to soil for cultivation, harvest their seeds, and obtain T2 generation.

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Determination of Arabidopsis taproot

To measure root length, *Arabidopsis* seeds were cleaned and disinfected with T2 generation seed rinse solution for 30 s, washed 4 times with sterile water, and protected from light with aluminum foil, this ensures that the testing is conducted in a sterile and effective environment. Place them in an environment of 4°C for 72 h, place scattered or clustered spots on 1/2 MS solid medium, and place them in a Thermo Scientific Precision 818 large-capacity plant incubator for cultivation. Measure the length of the main root after the seeds germinate, draw the growth curve and calculate the root length growth rate. The observation process uses a microscope to set the objective magnification to 20 times for observing and recording the root zone. The specific calculation formula of the root length growth rate (RLG) is shown in *Equation 1*.

$$RLG = \frac{LabRL - ConRL}{ConRL} \times 100\% \quad \text{(Eq.1)}$$

In *Equation 1*, *LabRL* represents the length of the main root of the experimental group, and *ConRL* represents the length of the *ConRL* main *LabRL* root of the control group.

In order to measure the length and width of the root meristematic zone and count the number of cells in the main root at the same time, put the whole plant seedlings grown on 1/2 MS solid medium on a glass slide filled with a transparent solution, press the slices, and use DIC mode. Observe the root meristematic zone with an inverted microscope, take pictures and save them. The meristematic zone is located between the quiescent center and the first cells of the cortex, and the area where there is a marked change in lateral relationship is the meristem.

Observations on the localization of NGAL1 protein in roots

The fluorescent signal distribution in roots of the Ngal-Tri pNgal1-GFP strain Ngal-Tri grown on 1/2 MS medium for 5 days was observed by fluorescence microscope and laser confocal microscope. Subsequent Bi FC experiments.

Primers were designed to amplify the DNA fragments of NGAL1, TMO5 and LHW c, and these fragments were ligated into pUC18-p35S:YN and pUC18-p35S:YC transient expression vectors by enzyme digestion, respectively. Large-scale extraction of recombinant plasmids was performed to obtain high-purity, high-concentration plasmid DNA, and the fusion protein expression vector and empty vector pUC18-p35S:YN or pUC18-p35S:YC were used as controls. The preparation and transformation methods of protoplasts are as follows.

(1) Construction of Fluorescent Fusion Protein Expression Vector: Use wide type *Arabidopsis* thaliana cDNA and appropriate primers to amplify the fragment of the target gene, and use T4 DNA ligase to connect to the PTF486 horizontal expression vector to generate a recombinant fusion protein expression vector.

(2) Preparation and transformation of *Arabidopsis* protoplasts: The leaves of *Arabidopsis* thaliana grown for 3-4 weeks were cut into strips, placed in the enzyme digestion solution for 3 h, and placed on a horizontal shaker at 40 rpm for 3 min. Remove the supernatant, resuspend the cells in 10 mL of W5 solution, and centrifuge at 100 g for 2 min at 4°C. Remove the supernatant and resuspend the cells in 10 mL of W5 solution. After 40 min on ice, remove the supernatant. Add an appropriate amount of MMg solution to make the number of cells in the solution about 2×105/mL. An equal

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volume of 40% PEG solution was added, mixed gently and left at room temperature for 23 min. Then the transformed protoplasts were resuspended in a hexagonal plate containing 1 mL of W5 solution, and cultured slowly at 22°C and 40 rpm on a horizontal shaker in the dark for 12 h, and then the fluorescent signals were observed and photographed with a laser confocal microscope.

Statistical methods

SPSS23.0 and EXCEL software were used for postoperative data processing. The comparison between the two experimental groups was conducted using the chi square test, and the comparison of count data was conducted using the same method. $P < 0.05$ indicates statistical significance between the two sets of data.

Results

Ngal1-Tri pNgal1-GFP can better restore the root phenotype of Ngal1-Tri

Using tissue-specific analysis, we found that NGAL1 is expressed in roots, and that the shoots of triple Ngal1-Tri loss-of-function mutants have an enlarged leaf margin phenotype, and that shoots grow faster than wild-type roots. In order to further study, the function of NGAL1 in root development, the binary vector was transformed into Ngal1-Tri to generate Ngal-Tri pNgal1-GFP transgenic inversion. The observation of the root length of the Ngal-Tri pNgal1-GFP reduction system and the Ngal1-Tri function loss triple mutant showed that after 5 d of growth, the main root of Ngal1-Tri was significantly elongated compared with the wild type, while the Ngal-Tri pNgal1-There was no significant change in root length in the GFP reduction system compared with WT. From 48 h, the root length was measured by visualization for 24 h, and the root length growth curve was drawn. It can be seen that the root length elongation of Ngal1-Tri from seed germination was significantly faster than that of WT, while that of Ngal1-Tri. The growth curve of NGAL1-GFP basically coincided with that of the wild type, indicating that the function of NGAL in regulating root development is redundant, and that upregulation of NGAL1 expression is sufficient to restore root length of Ngal1-Tri in the Ngal1-Tri background. *Figure 2* shows the straight root growth curves of three types of seeds.

Observing *Figure 2*, it can be concluded that the loss of NGALs function will lead to an increase in the length of the main root of *Arabidopsis*. Taproot growth curves of Wide Type, Ngal1-Tri, Ngal1-Tri pNgal1-GFP seeds from 48 h to 168 h after germination. The sample size of each data is 100.

Ngal1-Tri, which has acquired the ability to express the transcription factor NGAL1, to restore the root phenotype of the Ngal1-Tri triple loss of function mutant, the Wide Type, Ngal1-Tri, Ngal1-Tri pNgal1-GFP seedlings that had germinated for 72 h were taken out, and Take the transparent liquid to process it, and observe the length of the root meristematic zone. Count the length and width of the main root meristematic zone of Wide Type, Ngal1-Tri, Ngal1-Tri pNgal1-GFP. *Figure 3* shows the statistical data of meristem length and width for three types of seeds after 72 h of growth.

Observing *Figure 3*, it can be seen that the length of the meristem of Ngal1- Tri is significantly increased compared with that of Wide Type, which is mainly due to the increase in the number of cells in the meristem of the cortex of Ngal1-Tri, and the width of the meristem of the root also increased. The sample size of each data is 50. *Figure 4*

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shows the statistical data of the number and average width of meristem cells in three types of seeds after 72 h of growth.

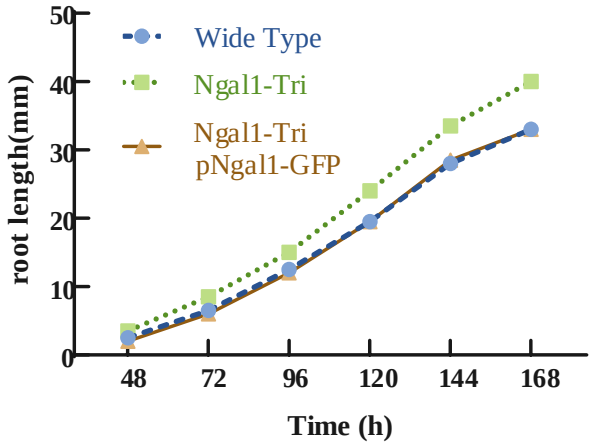

Figure 2. *Taproot growth curves of three kinds of seeds*

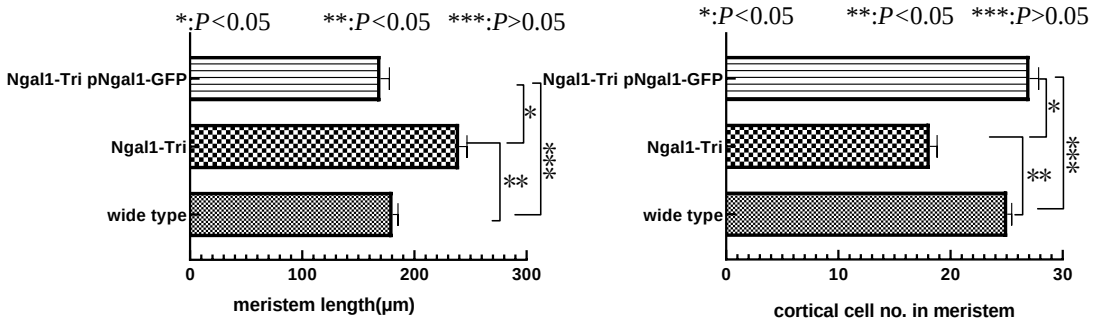

Figure 3. *Statistical data of meristem length and width of three kinds of seeds grown for 72 h*

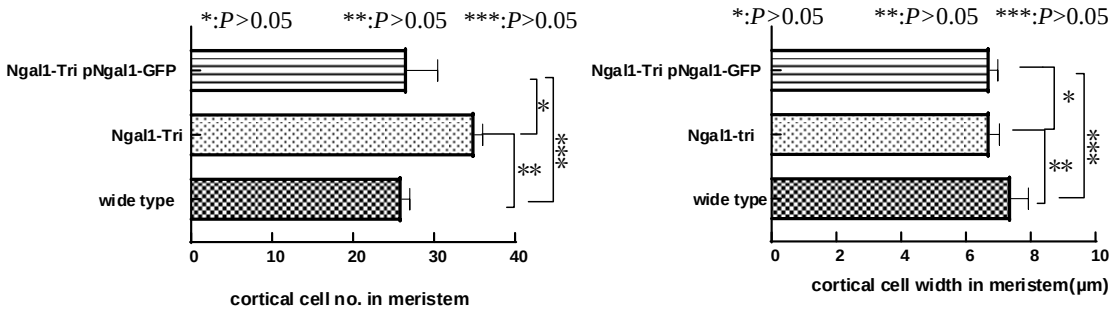

Figure 4. *Statistical data of meristem cell number and average width of three kinds of seeds grown for 72 h*

Observing *Figure 4*, it can be seen that the length and width of the meristematic zone of Ngal1-Tri pNgal1-GFP are reduced compared with Ngal1-Tri, while the average width of the meristematic zone cells of the seedlings cultured by the three kinds of seeds has no significant difference. The width of the living zone also increases. The sample size of each data is 100.

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Overexpression of NGAL1 gene leads to shorter main root length of Arabidopsis

In this study, in order to understand whether the NGAL1 transcription factor is related to the growth of *Arabidopsis* taproot, the transgenic lines NGAL1-p35s, wide type and loss-of-function mutant NHAL1-2 with overexpressed NGAL1 were selected in 1/2 MS solid medium cultivated on. Beginning 48 h after the *Arabidopsis* seeds germinated, the length of the main root of each group was measured every day, and the growth curve obtained is shown in *Figure 5*.

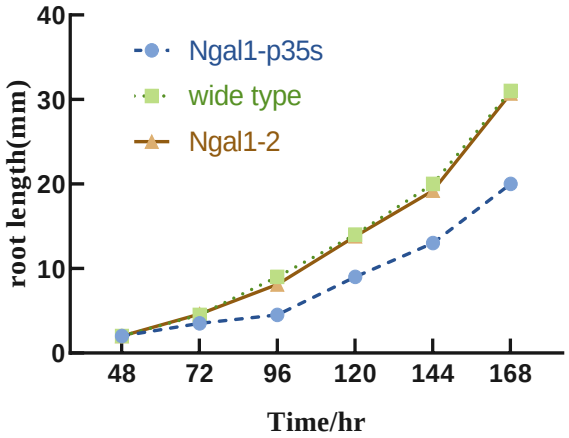

Figure 5. *Taproot growth curves of three kinds of seeds*

Observing *Figure 5*, it can be seen that the main root of Ngal1-p35s is obviously shorter than that of wide type, while the length of main root of Ngal1-2 is not significantly different from that of wide type. At the same time, it can be seen from *Figure 5* that the growth rate of the main root of Ngal1-p35s seeds at all-time points from 48 h to 168 h after germination was significantly lower than that of wide type, indicating that the transcription factor Ngal1 is involved in the regulation of *Arabidopsis* The role of taproot development, but the reason why the taproot length of Ngal1-2 *Arabidopsis* seedlings did not show changes may be caused by the duplication of NGALs gene functions. The sample size of each data is 100.

Experiment, in order to find the reason why the main root length of Ngal1-p35s seedlings became shorter, three kinds of seedlings 72 h after germination were treated with transparent liquid, and the length and width of their meristems and the total number of cells in the cortical meristems were measured. The experimental results are shown in *Figure 6*.

Observing *Figure 6*, it can be seen that the length of the meristematic zone of Ngal1-p35s seedlings is significantly shorter than that of the wide type, and the width of the meristematic zone is also significantly reduced. The sample size of each data is 100. *Figure 7* shows the number and average width of cells in the cortical meristem of three *Arabidopsis* seeds.

Observing *Figure 7*, it can be seen that the number of cells in the cortical meristem of Ngal1-p35s seedlings is significantly less than that of wide type seedlings, while the number of cells in the cortical meristem of Ngal1-2 is significantly more than that of wide type seedlings. However, there was no significant difference in the average width of cortical meristem cells of the three *Arabidopsis* seeds. It shows that the overexpressed transcription factor Ngal1 can reduce the number of cells in the root

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meristematic zone, which can also explain the reason why Ngal1-p35s shortens the length of the main root.

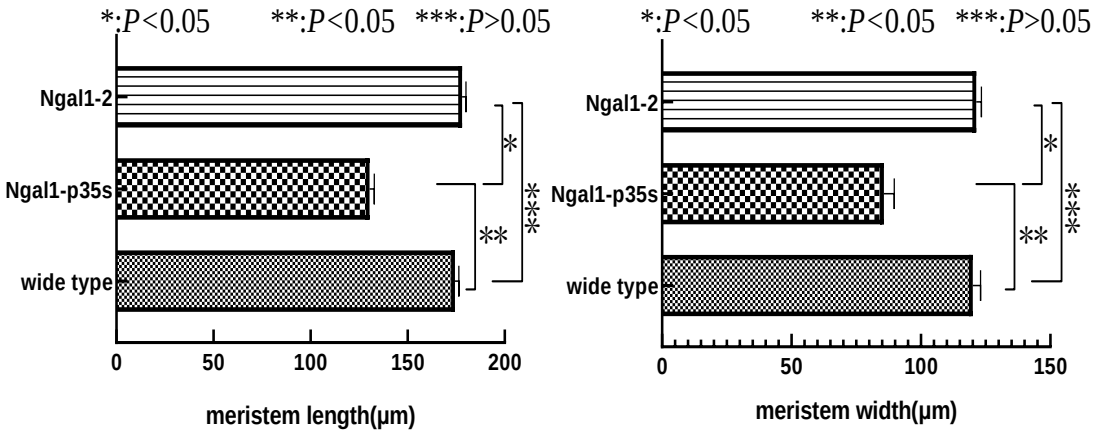

Figure 6. *Comparison of meristematic zone length and width of three kinds of Arabidopsis seeds*

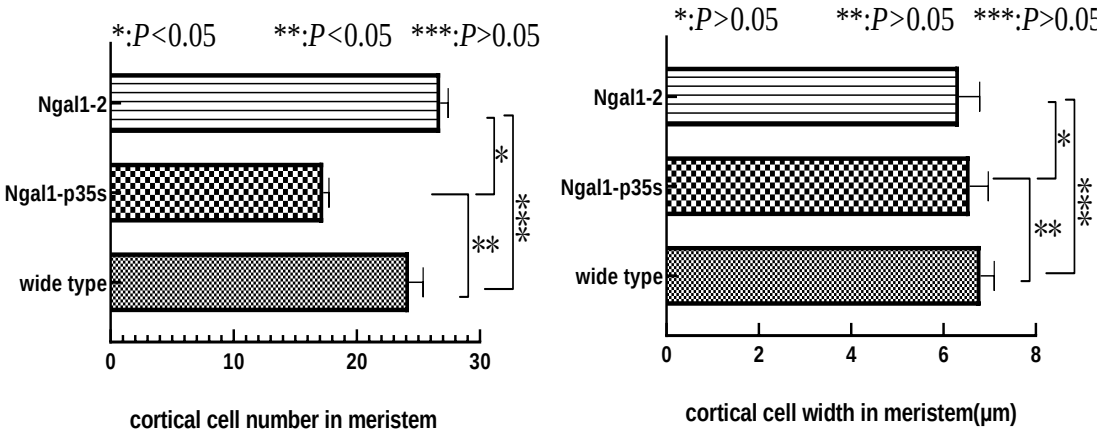

Figure 7. *Cell number and average width of cortical meristem of three kinds of Arabidopsis seeds*

There is a genetic interaction between NGALs and TMO5

To analyze the genetic relationship between the NGAL family of transcription factors and the plant vascular transcription factor TMO5, a quadruple loss-of-function mutant tmo5 Ngal1-Tri was used in this study. Four kinds of seeds, wide type, single mutant tmo5, triple mutant ngal1-Tri, and tmo5 Ngal1-Tri, were cultivated on 1/2 MS solid medium, and the seedlings were observed on the 7th day after seed germination, as shown in *Figure 8* result.

Observing *Figure 8*, it can be seen that the length of the main root of the tmo5 seedlings on the 4th and 7th day of germination was slightly increased compared with that of the wide type seedlings. Compared with the seedlings of the other three seeds of Ngal1-Tri, the length of the main root of the triple mutant Ngal1-Tri was significantly longer, while the length of the main root of the tmo5 Ngal1-Tri was shorter than that of the Ngal1-Tri, indicating that compared with the triple mutant it shows no tendency to

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taproot growth. At the same time, observing *Figure 8,* we can know that the length of the main root of the wide type is about 9.2 mm, the length of the main root of the tmo5 is about 10.8 mm, which is 17.3% higher than that of the wide type, and the length of the main root of the Ngal1-Tri is about 14.0 mm, an increase of 52.1% compared with the wide type, and the root length of tmo5 Ngal1-Tri was about 12.1 mm, an increase of 31.5% compared with the wide type, and there was no additive effect. Seven days after seed germination, the main root lengths of the four seedlings were measured and statistically analyzed. The results obtained were similar to the data obtained four days after germination, indicating that the plant vascular transcription factor TMO5 gene functions under the condition of Ngal1-Tri The deletion of Ngal1-Tri can suppress the phenotype of taproot length, and at the same time, it indicates that there is a genetic relationship between the transcription factor NGAL family and the plant vascular transcription factor TMO5. The sample size of each data is 100.

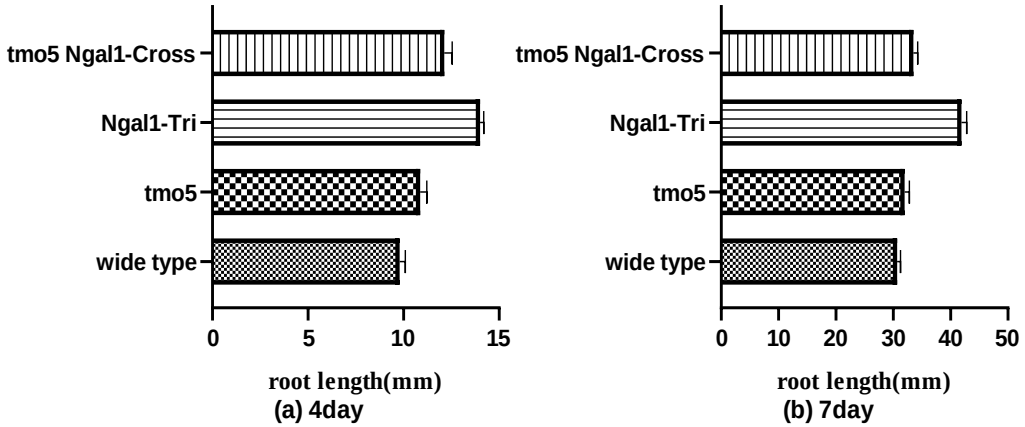

Figure 8. *The effect of the loss of the function of the plant vascular transcription factor TMO5 in the triple loss-of-function mutant Ngal1-Tri on the taproot length*

In this study, in order to understand whether the plant vascular transcription factor TMO5 cooperates with the transcription factor NGAL family to participate in the development of the root meristem, different genotypes of *Arabidopsis* thaliana 72 hafter germination were treated with transparent liquid, and the root tip meristem was measured. The length and width of the zone, the result shown in *Figure 9* is obtained.

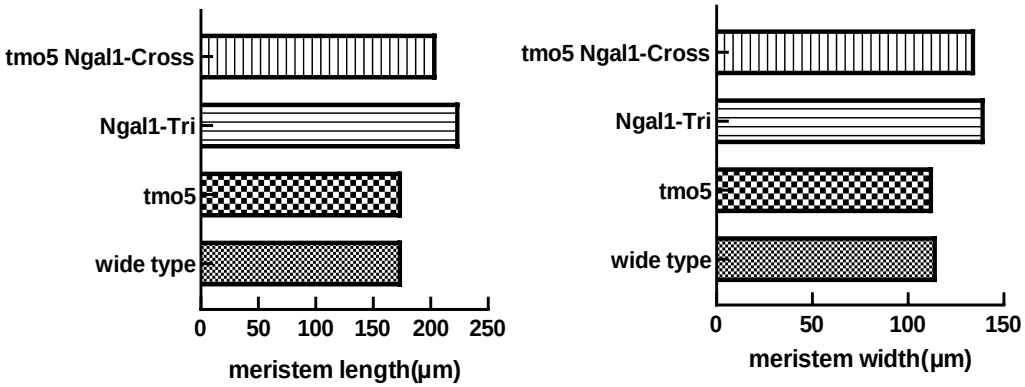

Figure 9. *The effect of the loss of the function of the plant vascular transcription factor TMO5 in the triple loss-of-function mutant Ngal1-Tri on the taproot length*

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Observing *Figure 9*, it can be seen that after 72 h of germination of the four seedlings, there is no significant difference in the length and width of the root tip meristem between the plant vascular transcription factor tmo5 and the wide type. Compared with the wide type, tmo5 Ngal1-Tri has both the length and width of the root tip meristematic zone increased. At the same time, it can be seen that under the Ngal-Tri background, because the plant vascular transcription factor tmo protein is partially missing, the growth of the root meristematic zone of the main root of Ngal-Tri is inhibited, resulting in an increase in its length and width. The sample size of each data is 100, and each measurement is an independent plant sample. The average value and retrograde analysis are selected, and the changes in the number of woven cells are shown in *Figure 10*. *Figure 10* shows the changes in the number of cells in root meristem tissue during the loss of function of plant vascular transcription factor TMO5 in the triple functional deficient mutant Ngal1 Tri.

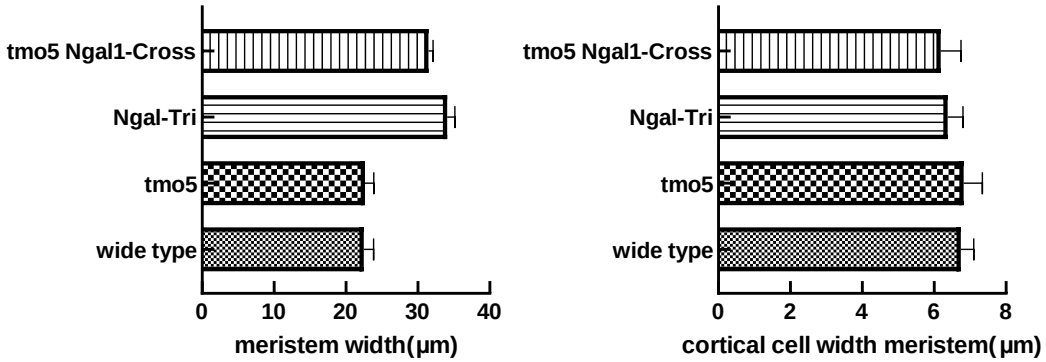

Figure 10. *Changes in the number of cells in the root meristem under the loss of function of the plant vascular transcription factor TMO5 in the triple loss-of-function mutant Ngal1-Tri*

Observing *Figure 10*, it can be seen that there is no significant difference between the plant vascular transcription factor tmo5 and the wide type in the total number of cells in the cortical meristem and its average width. Compared with the wide type, the cortical meristem of tmo5 Ngal1-Tri The number of cells increased. However, in the Ngal-Tri background, due to the partial deletion of the functional plant vascular transcription factor tmo5 protein, the total number of cells in the cortical meristem increased.

Discussion

NGALs are transcription factors in the B3 family of plants, mainly involved in regulating leaf edge development. NGALs (NGATHA LIKE subfamily transcription factors) play an important regulatory role in plant growth (Su et al., 2022). Overexpression of NGALs leads to the formation of cup-shaped cotyledons and smooth edged true leaves, while functional loss leads to an aggravated phenotype of dentate leaf edges, indicating that NGALs proteins are involved in the development of leaf edges. NGALs, as transcription factors, participate in regulating the development process of plant leaves, especially by affecting the expression of CUC2 genes to regulate the formation of leaf margin serrations (Tang et al., 2021). However, the mechanism of action of NGALs in plant roots is currently unclear. Therefore, studying the

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transcriptional effects of NGALs on *Arabidopsis* root systems is necessary to better understand the role of NGALs in plant growth and development (Vaseva et al., 2021).

In tissue-specific analysis, plants overexpressing Ngal1-p35s had significantly shorter root lengths than the wild-type main root, while the mutant Ngal1 Tri, which lost Ngal1 function, had significantly longer root lengths compared to the wild-type main root. This may be related to the functional redundancy of NGAL1, NGAL2, and NGAL3, leading to longer root lengths than the wide type natural root. The transgenic plant Ngal Tri-p NGAL1: NGALl GFP obtained by transferring the binary vector p Cambia1300-p NGAL1: NGAL1 GFP into Ngal Tri can better restore the abnormal root phenotype of the Ngal Tri triple function loss mutant, indicating the role of the Ngal family in root development.

In the following studies, in order to investigate the possible role of Ngal1 in root development, triple function loss mutants Wide Type, Ngal1 Tri, and Ngal1 Tri pNgal1 GFP seedlings were selected for experiments, and the length of their root meristematic zone was analyzed (Yan et al., 2021). The results showed that the meristematic length of Ngal1 Tri was significantly increased compared to Wide Type, which was mainly related to the increase in the number of cortical meristematic cells in Ngal1 Tri. In addition, in subsequent studies, the number and average width of meristematic cells after 72 h of seed growth were statistically analyzed. Among them, the length and width of the meristematic zone of Ngal1 Tri pNgal1 GFP were reduced compared to Ngal1 Tri, while there was no significant difference in the average width of meristematic zone cells among the three cultured seedlings. In addition, transgenic lines NGAL1-p35s with overexpression of NGAL1, as well as the broad and functionally deficient mutants NHAL1-2, were screened from 1/2MS and subjected to retrograde experiments. After 168 h of cultivation, the main root length of NGAL1-p35s was significantly shorter than that of Ngal-1-2 and Wide Type, indicating that the transcription factor Ngal1 may be involved in regulating *Arabidopsis* root development.

In addition, according to relevant studies, there is a genetic relationship between the transcription factor NGAL family and the plant vascular transcription factor TMO5, which affects plant roots (Yu et al., 2021). According to relevant literature research, overexpression of TMO5 leads to the early development and formation of the intermediate cortex. The early appearance of the intermediate cortex in ngaltri may be related to TMO5, and NGAL1-GFP is also distributed in the active expression sites of both. According to the experimental results, partial deletion of the plant vascular transcription factor tmo protein can lead to inhibition of growth in the root meristematic zone of Ngal Tri. At the same time, the loss of TMO5 function in plant vascular transcription factors to some extent weakens the phenotype of root elongation and thickening associated with ngaltri triple functional loss mutations.

Based on the above research, it can be found that transcription factors NGALs are expressed in the main roots of *Arabidopsis* thaliana. Compared with the wild type, the ngaltri triple functional deletion mutant showed longer main roots and earlier development of the root cortex. In addition, NGAL1 can interact with the root development related gene encoding protein TMO5 to participate in the regulation of root development. It can be seen that transcription factors NGALs play an important role in the regulation of plant roots, and are of great significance for the study of plant growth and development.

Conflict of interests. The author declares that there is no conflicting interest in this research.

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