Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Functional Study on the Regulation of Adventitious Root Formation by Serine Acetyltransferase 4 in Peach Rootstock ‘GF 677’

Xuebing Zhang

460332042@qq.com

CAAS Gansu Academy of Agricultural Sciences

Lanlan Hao

CAAS Gansu Academy of Agricultural Sciences

Fan Zhang

CAAS Gansu Academy of Agricultural Sciences

Hong wang

CAAS Gansu Academy of Agricultural Sciences

Research Article

Keywords: Peach rootstock ‘GF677’, gene cloning, adventitious root formation, genetic transformation, functional verification

Posted Date: July 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9970137/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Objective

To investigate the molecular characteristics and biological function of *PpSAT4* (a serine acetyltransferase-encoding gene) in adventitious root formation, and explore its potential as a candidate gene for improving the rooting ability of difficult-to-root peach rootstocks via molecular breeding.

Methods

PpSAT4 was identified from the adventitious root transcriptome of the peach rootstock ‘GF677’. Its function in adventitious root formation was validated through heterologous overexpression in *Prunus salicina* and *Arabidopsis thaliana*.

Results

PpSAT4 encodes a 287-amino-acid protein, with a predicted molecular weight of 30.49 kDa and an isoelectric point (pI) of 6.70. Promoter sequence analysis identified multiple cis-acting regulatory elements, including light-responsive, auxin-responsive (e.g., TGA-element) and gibberellin-responsive (e.g., P-box) elements, indicating that *PpSAT4* expression may be regulated by key plant hormones and environmental signals during adventitious root initiation. Stable *PpSAT4*-overexpressing transgenic lines were successfully obtained in both species. In *Prunus salicina*, *PpSAT4* overexpression significantly promoted adventitious root formation (increased root length, root surface area, root diameter, root volume and root tip number) and enhanced root peroxidase (POD) and superoxide dismutase (SOD) activities. Consistent results were observed in transgenic *Arabidopsis*, which showed longer primary roots, higher lateral root density, and elevated POD and SOD activities. These results confirm the conserved pro-rooting function of *PpSAT4* in peach.

Conclusion

PpSAT4 positively regulates adventitious root formation, likely by modulating redox homeostasis. It is a promising target for improving rooting capacity of difficult-to-root peach rootstocks through genetic engineering.

1. Introduction

The use of clonal rootstocks instead of seedling rootstocks has become a common practice in peach (*Prunus persica*) nursery production, and cutting survival rate largely depends on adventitious root formation [1]. However, adventitious root development is relatively difficult in woody plants such as peach, and the rooting process is regulated by multiple factors [2, 3]. In recent years, research on

adventitious root formation via cuttings has encountered a bottleneck that cannot be resolved by physiological studies alone. Therefore, investigating the characteristics and functions of key genes involved in adventitious root development in peach will not only help elucidate the molecular mechanism of rooting, but also improve the regulatory network underlying adventitious root formation, which is of great practical significance for accelerating clonal propagation of peach trees.

Adventitious root formation is indispensable for the clonal propagation of horticultural crops with high economic value. Clonal propagation is commonly employed in breeding programs due to its advantages of short growth cycle and high propagation efficiency. However, the failure of stem cuttings to form adventitious roots remains a major bottleneck limiting cutting survival [4–6]. *Peter* et al. [7] reported that high activities of peroxidase (POD) and superoxide dismutase (SOD) are beneficial to adventitious root induction. After the cutting propagation of hybrid larch, root tissues were collected at five time points for enzyme activity assays. The results showed that POD, indoleacetic acid oxidase, and polyphenol oxidase play important roles in the rooting process of larch cuttings [8]. In recent years, more and more studies have focused on the molecular mechanisms underlying plant adventitious root formation, particularly the regulatory roles of gene networks. During adventitious root development in Arabidopsis explants, wounding-induced auxin activates the expression of WOX11 and WOX12, which in turn upregulate LBD16 and LBD29; these genes further directly activate WOX5 and WOX7, thereby facilitating adventitious root initiation [9, 10]. *Liu* et al. [11] demonstrated that peu-miR160a negatively regulates the expression of PeARF10.1, PeARF16.1, PeARF16.2, PeARF16.3, PeARF17.1, and PeARF17.2 in poplar. Overexpression of PeARF17.1 or mPeARF17.2 significantly increases the number of adventitious roots in cuttings. In summary, adventitious root formation in plants is a complex physiological process regulated by multiple genes and signaling pathways [12], and further exploration of its molecular mechanisms is of great significance for optimizing clonal propagation of horticultural crops.

Previous studies have shown that Serine Acetyltransferase (SAT) is involved in plant growth and development as well as environmental adaptation processes. Under abiotic stress conditions, chloroplast-localized SISATs serve as the main response factors, and SISERATs adopt different strategies to cope with osmotic stress, ionic toxicity, and other stresses [13]. *Rajab* et al. [14] found in Brassica napus that NtSAT4 transgenic lines could overproduce high levels of cysteine and glutathione, thereby enhancing stress resistance, especially tolerance to heavy metals. In the identification of the SAT gene family in Camellia sinensis, *Wang* et al. [15] observed that the expression level of CssSAT1 increased significantly under drought stress, indicating a potential increase in its sensitivity to drought. In contrast, CssSAT2 showed a significant upregulation as early as 8 h, suggesting a more rapid response. Meanwhile, the expression level of CssSAT3 increased significantly at 48 h, indicating its potential role in long-term drought adaptation. Under salt stress, the expression level of CssSAT1 was significantly upregulated at 48 h, implying that it may be involved in the later adaptation mechanism to salt stress. Currently, most studies on SAT have focused on abiotic stress and metal stress [15–17], while studies on their role in adventitious root formation of fruit trees are rarely reported.

In this study, the peach rootstock ‘GF677’ was used as the test material. Based on the previous transcriptome sequencing data of the research group, the PpSAT4 related to adventitious root formation was screened and cloned, and its function was identified. As a woody plant, peach is difficult to achieve successful genetic transformation. However, plum, as a close relative of peach, has a similar genetic background. Therefore, plum was used for genetic transformation in this experiment to explore the function of PpSAT4 in adventitious root formation. In addition, Arabidopsis thaliana, one of the most commonly used model plants for gene function research, was also selected for transformation. The function of PpSAT4 in adventitious root development was analyzed by combining the phenotypic observation and index detection of plum and Arabidopsis thaliana, so as to provide a theoretical basis for exploring the molecular mechanism of adventitious root development in peach.

2. Results

2.1 Physicochemical properties, hydrophobicity/hydrophilicity, and secondary and tertiary structure analyses of *PpSAT4*

Based on our group’s previously obtained transcriptome data (unpublished), the *PpSAT4* gene associated with adventitious root formation was screened and cloned. Analysis of physicochemical properties showed that *PpSAT4* encodes a protein of 287 amino acids, with a theoretical molecular weight of 30.49 kD and a theoretical isoelectric point (pI) of 6.70, indicating a weakly acidic protein. Subcellular localization prediction suggested that the protein may be distributed in the cytoplasm (7%), chloroplast (2%), cytoskeleton (2%), mitochondria (1%), and cell membrane (1%), implying primary localization in the cytoplasm.

Prediction of the secondary structure revealed that the PpSAT4 protein is mainly composed of α-helix, extended strand, and random coil (Fig. 1a). Among these, α-helix is the dominant structural unit, particularly concentrated in the N-terminal region. Extended strands are mainly located in the central region of the protein, whereas random coils are distributed throughout the entire sequence, forming flexible domains of the protein. Hydrophobicity analysis indicated that the grand average of hydropathicity of the PpSAT4 polypeptide chain was greater than 0 (Fig. 1b). Multiple highly hydrophobic regions were observed across the sequence, suggesting that PpSAT4 functions as a hydrophobic protein.

2.2 Homology alignment and phylogenetic tree analysis of *PpSAT4*

Homologous sequences of *PpSAT4* were retrieved from the NCBI database, and multiple sequence alignment of SAT4 proteins from diverse plant species was performed using DNAMAN software. The results showed that the N-terminal region of the PpSAT4 protein exhibited certain species-specific

variations, whereas the C-terminal region was highly conserved and contained multiple conserved domains shared by homologous proteins (Fig. 2a). This finding suggests that the C-terminal region may be essential for maintaining the core function of the protein.

A phylogenetic tree of SAT4 proteins from different species was constructed using MEGA-X software based on the neighbor-joining (NJ) method. Phylogenetic analysis revealed that *PpSAT4* was first clustered with homologous proteins from *Prunus dulcis* and *Prunus avium* (both belonging to the same genus *Prunus*) indicating the closest genetic relationship and highest sequence similarity. It was then grouped into a major clade with homologs from other Rosaceae species including apple and rose. In contrast, PpSAT4 showed relatively distant phylogenetic relationships with homologs from non-Rosaceae species, such as *Jatropha curcas* (Euphorbiaceae), *Abrus precatorius* and *Cajanus cajan* (Fabaceae), and *Momordica charantia* (Cucurbitaceae) (Fig. 2b).

2.3 Cis-acting regulatory elements analysis of *PpSAT4*

Prediction and analysis of cis-acting regulatory elements in the upstream regulatory sequence of *PpSAT4* revealed that its promoter region contains various key elements associated with light responsiveness and hormone responses (Table 1). These elements include light-responsive G-box, gibberellin-responsive P-box, auxin-responsive TGA-element, and abscisic acid-responsive ABRE. These observations suggest that the expression of *PpSAT4* may be coordinately regulated by light signals and multiple plant hormones, including gibberellin, auxin, and abscisic acid. Collectively, these findings imply that *PpSAT4* is likely involved in the integration and transduction of hormonal and environmental signals during the process of adventitious root initiation and development.

Table 1
Important cis-acting regulatory elements in the regulatory sequences of *PpSAT4*

Regulatory sequence	**sequence**	**Function of site**
G-box	G-Box	*cis*-acting element involved in light responsiveness
P-box	CCTTTTG	gibberellin-responsive element
TGA-element	AACGAC	auxin-responsive element
ABRE	CACGTG	*cis*-acting element involved in the abscisic acid responsiveness

2.4 Determination of phenotypic and morphological indexes in transgenic plum (*Prunus salicina*)

To further investigate the biological function of *PpSAT4* during adventitious root formation in plum, a *PpSAT4* overexpression vector was constructed and introduced into *Prunus salicina* via Agrobacterium-mediated transformation, generating multiple candidate transgenic lines. Genomic DNA-based PCR identification showed that specific bands of the expected size were amplified in overexpression lines L1,

L2, L3, L4 and L5, whereas no corresponding band was detected in wild-type (WT) plants, indicating that exogenous *PpSAT4* was successfully integrated into the plum genome (Fig. 3a). Total RNA was extracted from transgenic lines and WT plants, and the expression level of *PpSAT4* was analyzed by Quantitative Real-Time PCR (qRT-PCR). Compared with WT, the transcript abundance of *PpSAT4* was extremely significantly upregulated in lines L1, L3 and L4 ($P < 0.05$), confirming the successful generation of *PpSAT4*-overexpressing transgenic materials (Fig. 3a). Therefore, three high-expression lines L1, L3 and L4 were selected for subsequent functional analysis.

Tissue-cultured seedlings of WT and overexpression lines with consistent growth status were cut and cultured on solid MS medium. After 45 days of culture, phenotypic observation revealed that plants overexpressing *PpSAT4* exhibited significantly higher root indices and more developed root systems compared with WT (Figs. 3b, c). To quantitatively evaluate the effect of *PpSAT4* overexpression on root morphogenesis, root morphological parameters of WT and transgenic lines were measured using a root scanner (Fig. 3d). The results showed that root length, root surface area, root diameter, root volume and root tip number of WT plants were relatively low, whereas all these parameters were significantly higher in *PpSAT4* overexpression lines. These results demonstrate that overexpression of *PpSAT4* significantly promotes the initiation, elongation and branching of adventitious roots in plum, and acts as a positive regulator of adventitious root formation. The promoting effect is mainly reflected in increases in root length, root surface area, root volume and root tip number, with no significant influence on average root diameter.

2.5 Determination of enzyme activities in roots of transgenic *Prunus salicina*

To investigate the effect of *PpSAT4* overexpression on the plant antioxidant system, the activities of POD and SOD were measured in roots of WT and *PpSAT4*-overexpressing lines (L1, L3, L4). The results showed that POD and SOD activities were significantly higher in overexpression lines than in WT plants ($P < 0.05$).

Specifically, POD activity increased by approximately 16–19% and SOD activity increased by 18–23% in overexpression lines compared with the WT (Fig. 4). These results indicate that overexpression of *PpSAT4* significantly enhances the activities of antioxidant enzymes and improves the ability to scavenge reactive oxygen species, which may represent one of the physiological mechanisms underlying the promotion of adventitious root growth and development by *PpSAT4*.

2.6 Determination of phenotype and enzyme activity in transgenic *Arabidopsis thaliana*

To further verify the function of *PpSAT4*, an overexpression vector was constructed and transformed into *Arabidopsis thaliana*. Genomic PCR identification showed that specific bands consistent with the target fragment (864 bp) were amplified in lines L1-L5, whereas no corresponding band was detected in WT

plants, indicating that exogenous *PpSAT4* was successfully integrated into the *Arabidopsis* genome (Fig. 5a). qRT-PCR analysis revealed that the expression levels of *PpSAT4* in lines L1, L3 and L4 were significantly higher than those in WT, so these three lines were selected for subsequent phenotypic and physiological analysis.

Wild-type and transgenic *Arabidopsis* seedlings were cultured on MS solid medium for 10 days, and robust and uniform plants were selected for analysis. Compared with the WT, primary root growth was significantly promoted in *PpSAT4* overexpression lines, with more developed root systems (Fig. 5b). Statistical analysis of primary root length showed that root lengths of L1, L3 and L4 were significantly longer than that of WT ($P < 0.05$) (Fig. 5c).

To explore the effect of *PpSAT4* overexpression on the antioxidant system of *Arabidopsis*, antioxidant enzyme activities in roots were further measured. The results showed that POD and SOD activities were significantly increased in overexpression lines compared with the WT ($P < 0.05$). Specifically, POD activity increased by approximately 15–20% and SOD activity by 12–18% (Figs. 5d, e). These results indicate that overexpression of *PpSAT4* in *Arabidopsis* significantly promotes primary root growth and enhances antioxidant enzyme activities, which is consistent with the functional results in plum, further confirming the positive regulatory role of *PpSAT4* in adventitious root development.

3. Discussion

As a rate-limiting enzyme in cysteine biosynthesis, SAT is involved in various plant developmental processes and adaptations to environmental stimuli [18, 19]. To date, only a few members of the *SAT* gene family have been functionally characterized in Arabidopsis [20], Maize [21], and soybean [22], with few detailed reports available in other plant species. Therefore, in this study, the peach clonal rootstock 'GF677' was used as experimental material, and the *PpSAT4* gene, which responds to adventitious root formation, was screened and cloned based on transcriptome data. This gene encodes a protein of 287 amino acids with a molecular mass of 30.49 kD and an isoelectric point of 6.70, indicating an acidic protein. According to previous reports, a grand average of hydropathicity value greater than 0 indicates a hydrophobic protein, whereas a negative value indicates a hydrophilic protein [23]. On this basis, PpSAT4 was classified as a hydrophobic protein. In addition, subcellular localization prediction suggested that PpSAT4 is widely distributed in the cytoplasm and chloroplasts. Phylogenetic analysis showed that *PpSAT4* from peach is most closely related to its homologs in *Prunus dulcis* and *Prunus avium*, with the highest sequence similarity. Previous studies have suggested that SAT enzymes are regulated by the crosstalk of multiple plant hormones. For instance, in rice, expression profiles of *OsSAT* genes across different tissues, organs, and hormone treatments revealed that jasmonic acid acts as the main inducer of *OsSAT1;1*, *OsSAT2;1*, and *OsSAT2;2*, whereas auxin and abscisic acid specifically trigger the expression of *OsSAT1;1* [24]. In this study, analysis of the 2000-bp promoter region of *PpSAT4* revealed numerous hormone-related cis-acting regulatory elements, implying that this gene may participate in plant growth and development as well as hormone response pathways.

In this study, the *PpSAT4* gene was successfully cloned, and its overexpression vector was constructed. Via Agrobacterium-mediated transformation, transgenic *Prunus salicina* (a close relative of peach) and Arabidopsis thaliana plants were obtained, in which the transcription level of *PpSAT4* was significantly elevated compared with the WT. Previous research demonstrated that overexpression of the serine acetyltransferase gene *SlSAT* in tomato (*Solanum lycopersicum*) significantly promoted plant growth and development [13]. Similarly, genetic transformation studies in soybean [25], watermelon [26], spinach [27], and onion [28] showed that *SAT*-overexpressing plants exhibited markedly improved growth performance relative to WT plants. In the present experiment, root morphological indices as well as POD and SOD activities in transgenic plum and Arabidopsis were significantly higher than those in WT plants, indicating that overexpression of P*pSAT4* promotes adventitious root formation, enhances root system performance, and may improve survival rates after transplanting. *SAT* genes form a small family coordinately regulated by auxin and JA. Notably, within 1 hour of explant induction, prior to the upregulation of auxin-related genes, multiple JA-responsive genes are significantly induced [29]. Furthermore, crosstalk may exist between auxin and JA signaling pathways, which modulates the recognition of regulatory factors and subsequent transcriptional cascades, thereby contributing to adventitious root initiation and development [30]. Studies in Populus ussuriensis found that endogenous methyl jasmonate specifically induces strong upregulation of *PuHox52* during adventitious root formation. Plants overexpressing *PuHox52* exhibited altered adventitious root number and length, significantly increased root biomass, and rooting time advanced by 1–2 days compared with WT, confirming its role in promoting adventitious root development [31]. This is largely consistent with our results showing significantly increased root biomass in *PpSAT4*-overexpressing lines.

4. Materials and methods

4.1 Bioinformatics analysis of the *PpSAT4* gene

The physicochemical properties and hydrophobicity/hydrophilicity of the encoded protein were predicted using the ExPASy online tools (https://web.expasy.org/protparam/ and https://web.expasy.org/protscale/). Subcellular localization was predicted using WoLF PSORT (https://wolfpsort.hgc.jp/). The secondary structure of the protein was predicted with the NPS@ online server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html). The three-dimensional structure of the protein was predicted using the Phyre2 online tool (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi). Homologous amino acid sequence alignment was performed using DNAMAN software, and a phylogenetic tree was constructed using the NJ method. Cis-acting regulatory elements were analyzed using the PlantCARE online server (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

4.2 Gene cloning of PpSAT4

Primers were designed using Primer Premier 6.0 software **(Table S1)**. PCR amplification was performed using cDNA from the phloem of peach rootstock ‘GF677’ as the template. The PCR reaction system (40

μL) consisted of: 2× P515 polymerase mix 20 μL, 1 μL each of forward and reverse primers, 3 μL cDNA template, and 15 μL ddH_2O. The PCR program was set as follows: pre-denaturation at 95℃ for 5 min; 40 cycles of denaturation at 95℃ for 30 s, annealing at 58℃ for 30 s, and extension at 72℃ for 60 s; followed by a final extension at 72℃ for 10 min. The target band was then detected by electrophoresis and recovered.

4.3 Construction of plant expression vector and agrobacterium transformation

The vector CAM-FLAG was linearized using the restriction enzymes EcoRI and XhoI, and the purified target gene fragment was ligated to the linearized vector. The recombinant plasmid was then transformed into Escherichia coli DH5α competent cells. Positive monoclonal colonies were identified and sequenced for verification. The confirmed overexpression vector was transformed into Agrobacterium tumefaciens strain GV3101. Transformed cells were cultured upside down in a constant-temperature incubator at 28℃ until single colonies appeared, which were then verified by colony PCR.

4.4 Genetic transformation of *Prunus salicina*

Genetic transformation of plum (*Prunus salicina*) was performed according to the method described by previous study [32]. Mature, plump seeds were selected and decoated using a nutcracker. The seeds were sterilized in 1% sodium hypochlorite solution containing approximately 20 μL Tween-20 per 100 mL for 30 min with intermittent shaking. After rinsing 2–3 times, the seeds were soaked in sterile distilled water overnight at room temperature. The seed coat was then removed with a scalpel, and the radicle and epicotyl were discarded. Hypocotyls were cut into approximately 3 cross-sections (0.5-1 mm thick) and inoculated with Agrobacterium for 20 min. Hypocotyl segments were placed on antibiotic-free 3/4 MS regeneration medium for 3 days of dark culture, then transferred to regeneration medium supplemented with 250 mg/L cefotaxime and 30 mg/L kanamycin. After 6–8 weeks, newly formed shoots were transferred to MS growth medium containing antibiotics and cultured for about 3 weeks. Green, viable shoot clusters were excised, labeled, and subcultured onto fresh growth medium with antibiotics every 3 weeks. When shoots reached 3–4 cm in height, they were transferred to 1/2 MS rooting medium supplemented with antibiotics. Genomic DNA was extracted from leaves of putative transgenic plum plants, and transgene integration was verified by qRT-PCR.

4.5 Acquisition and genetic transformation of *Arabidopsis thaliana*

The harvested T_0 seeds were treated with 75% ethanol for 5 min and 26% sodium hypochlorite for 10 min, then rinsed 3–4 times with deionized water. Seeds were plated on medium containing 30 mg/L kanamycin, and resistant plants were screened by PCR. After three successive rounds of selection, T_3 homozygous transgenic lines were obtained.

4.6 qRT-PCR Analysis

First-strand cDNA was synthesized using a reverse transcription kit (TaKaRa, Dalian, China). Actin was used as the internal reference gene, and the corresponding primers are listed in **Table S1**. The reaction mixture (20 μL) contained: TB Green Premix Ex Taq II 10 μL, forward primer 0.8 μL, reverse primer 0.8 μL, cDNA template 2 μL, and ddH_2O 6.4 μL. The amplification program was as follows: pre-denaturation at 95℃ for 30 s; 40 cycles of denaturation at 95℃ for 10 s, annealing at 60℃ for 30 s, and extension at 72℃ for 30 s. Three biological replicates were performed for each sample.

4.7 Determination of Morphological Indexes and enzyme activities in transgenic lines

Rooted seedlings of three *PpSAT4*-overexpressing lines (L1, L3, L4) and one WT line of both plum and Arabidopsis were used for analysis. Ten plants per line were sampled with three biological replicates. Roots were excised and rinsed thoroughly with running water. Root systems of plum plants were scanned using a ScanMaker i800 Plus root scanner, and root architecture parameters including root length, surface area, root diameter, root volume, and root tip number were recorded. For Arabidopsis seedlings, primary root length was measured with a ruler, and phenotypic characteristics were photographed. Activities of SOD and POD were determined following the manufacturer's instructions provided by the enzyme-linked reagent kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China).

4.8 Data Analysis

Data processing and graphing were performed using Office 2021 and Origin 2021. Analysis of variance (ANOVA) was conducted using SPSS 22.0 software. Statistical significance was determined by one-way comparison at the $P = 0.05$ level.

5 Conclusion

The *PpSAT4* gene was successfully cloned from the peach rootstock 'GF677'. Analysis of cis-acting regulatory elements revealed that the promoter region of PpSAT4 harbors multiple responsive elements including those for auxin, abscisic acid, and gibberellin, indicating that PpSAT4 can respond to various external signals and may participate in plant growth and development as well as hormonal regulation. Genetic transformation assays showed that overexpression of PpSAT4 significantly increased multiple root morphological indexes (root length, root volume, root surface area, and root tip number) as well as POD and SOD activities in transgenic plum. Moreover, overexpression of this gene in *Arabidopsis thaliana* also markedly promoted primary root growth and enhanced antioxidant enzyme activities, suggesting that PpSAT4 may function similarly in Arabidopsis as in plum, which will be further verified in subsequent experiments. Collectively, our results preliminarily confirm that *PpSAT4* plays a positive role in promoting adventitious root formation. Further research will be conducted to dissect the molecular mechanism underlying *PpSAT4*-mediated adventitious root growth in peach, so as to provide a theoretical basis for the genetic improvement of peach, a woody species with low cutting survival rate.

Declarations

Conflicts of Interest:

The authors declare no conflicts of interest.

Ethics, Consent to Participate, and Consent to Publish declarations

not applicable.

Funding:

This research was supported with projects the National Natural Science Foundation of China Project (No. 32460734).

Data Availability Statement:

not applicable.

References

1. Uur R (2025) Evaluation of Suitable Rootstock and Key Criteria for Rootstock Breeding in Prunus Species. Appl Fruit Sci 67(6):475
2. Uwe D, Philipp F, Hajirezaei MR (2016) Plant Hormone Homeostasis, Signaling, and Function during Adventitious Root Formation in Cuttings. Front Plant 7(133):381
3. Tsipouridis C, Thomidis T, Michailides Z (2005) Factors influencing the rooting of peach GF677 (peach x almond hybrid) hardwood cuttings in a growth chamber. New Z J Experimental Agric 33(2):93–98
4. Zhang F, Ren J, Wang C (2025) Multi-omics analysis reveals the mechanism of adventitious roots formation in peach green branch cuttings. Front Plant Sci 16:1740503
5. Gong Y, Anees Arif M, Xu X, Lei M, Uwamungu JY, Wang S, Wang H (2025) Insights into Asexual Propagation Techniques and Molecular Mechanisms Underlying Adventitious Root Formation in Apple Rootstocks. Horticulturae 11(11)
6. Karapatzak E, Papanastasi K, Kyrkas D, Yfanti P, Nikisianis N, Maloupa E, Patakioutas G, Krigas N (2023) Asexual propagation for sustainable utilization of Greek indigenous germplasm of common dogwood (Cornus sanguinea L.), a neglected but noteworthy ornamental and nutraceutical species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*
7. Roussos PA (2023) Adventitious Root Formation in Plants: The Implication of Hydrogen Peroxide and Nitric Oxide. Antioxid (Basel Switzerland) 12(4)
8. Qin R, Zhao Q, Gu C, Wang C, Zhang L, Zhang H (2023) Analysis of oxidase activity and transcriptomic changes related to cutting propagation of hybrid larch. Sci Rep 13(1):1354

9. Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, Xu L (2014) WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in Arabidopsis. Plant Cell 26(3):1081–1093
10. Hu X, Xu L (2016) Transcription Factors WOX11/12 Directly Activate WOX5/7 to Promote Root Primordia Initiation and Organogenesis. Plant Physiol 172(4):2363–2373
11. Liu S, Yang C, Wu L, Cai H, Li H, Xu M (2020) The peu-miR160a-PeARF17.1/PeARF17.2 module participates in the adventitious root development of poplar. Plant Biotechnol J 18(2):457–469
12. Li S, Tahir MM, Wu T, Xie L, Zhang X, Mao J, Ayyoub A, Xing L, Zhang D, Shao Y (2022) Transcriptome Analysis Reveals Multiple Genes and Complex Hormonal-Mediated Interactions with PEG during Adventitious Root Formation in Apple. Int J Mol Sci 23(2)
13. Liu D, Li M, Guo T, Lu J, Xie Y, Hao Y, Wang L, Zhao D, Zhang L, Liu Z et al (2022) Functional characterization of the Serine acetyltransferase family genes uncovers the diversification and conservation of cysteine biosynthesis in tomato. Front Plant Sci 13:913856
14. Rajab H, Khan MS, Shah SH, Shah SMA (2019) Genetic Transformation of Tobacco Serine Acetyltransferase 4 (NtSAT4) gene in Brassica napus L. Sarhad J Agric 35(4)
15. Wang W (2024) The Serine Acetyltransferase (SAT) Gene Family in Tea Plant (Camellia sinensis): Identification, Classification and Expression Analysis under Salt Stress. Int J Mol Sci 25
16. Ahmad N, Malagoli M, Wirtz M, Hell R (2016) Drought stress in maize causes differential acclimation responses of glutathione and sulfur metabolism in leaves and roots. BMC Plant Biol 16(1):247
17. Fan C, Zou H, Zhang M, Jiang Y, Liu B, Sun Z, Su B (2025) Identification and Expression Analysis of the Soybean Serine Acetyltransferase (SAT) Gene Family Under Salt Stress. Int J Mol Sci 26(5):1882
18. Fan Z, Zhang S, Zhao J, Jia L, Yan C, Shu Y, Zhang K (2026) Identification of serine acetyltransferase (SAT) gene family and SAT2-mediated stress tolerance in cucumber, watermelon and melon. *Plant Science* 364(c):112945
19. Kurt F, Filiz E, Aydin A (2021) Bioinformatics Analyses of Serine Acetyltransferase (SAT) Gene Family in Rice (Oryza sativa) and their Expressions under Salt Stress
20. Howarth JR, Domínguez-Solís JR, Gutiérrez-Alcalá G, Wray JL, Romero LC, Gotor C (2003) The serine acetyltransferase gene family in Arabidopsis thaliana and the regulation of its expression by cadmium. Plant Mol Biol 51(4):589–598
21. Zhao Z, Li S, Ji C, Zhou Y, Li C, Wang W (2021) Genetic Variation of the Serine Acetyltransferase Gene Family for Sulfur Assimilation in Maize. Genes 12(3)
22. Fan C, Zou H, Zhang M, Jiang Y, Liu B, Sun Z, Su B (2025) Identification and Expression Analysis of the Soybean Serine Acetyltransferase (SAT) Gene Family Under Salt Stress. Int J Mol Sci 26(5)
23. Lv L, Xie Y, Li K, Hu T, Lu X, Cao Y, Zheng X (2018) Unveiling the Mechanism of Surface Hydrophilicity-Modulated Macrophage Polarization. Adv Healthc Mater 7(19):e1800675
24. Kurt F, Filiz E, Aydın A (2021) Genome-wide identification of serine acetyltransferase (SAT) gene family in rice (Oryza sativa) and their expressions under salt stress. Mol Biol Rep 48(9):6277–6290

25. Yi H, Dey S, Kumaran S, Lee SG, Krishnan HB, Jez JM (2013) Structure of soybean serine acetyltransferase and formation of the cysteine regulatory complex as a molecular chaperone. J Biol Chem 288(51):36463–36472
26. Saito K, Yokoyama H, Noji M, Murakoshi I (1995) Molecular cloning and characterization of a plant serine acetyltransferase playing a regulatory role in cysteine biosynthesis from watermelon. J Biol Chem 270(27):16321–16326
27. Noji M, Takagi Y, Kimura N, Inoue K, Saito M, Horikoshi M, Saito F, Takahashi H, Saito K (2001) Serine acetyltransferase involved in cysteine biosynthesis from spinach: molecular cloning, characterization and expression analysis of cDNA encoding a plastidic isoform. Plant Cell Physiol 42(6):627–634
28. McManus MT, Leung S, Lambert A, Scott RW, Pither-Joyce M, Chen B, McCallum J (2005) Molecular and biochemical characterisation of a serine acetyltransferase of onion, Allium cepa (L). Phytochemistry 66(12):1407–1416
29. Zhang G, Zhao F, Chen L, Pan Y, Sun L, Bao N, Zhang T, Cui CX, Qiu Z, Zhang Y et al (2019) Jasmonate-mediated wound signalling promotes plant regeneration. Nat plants 5(5):491–497
30. Bannoud F, Bellini C (2021) Adventitious Rooting in Populus Species: Update and Perspectives. Front Plant Sci 12:668837
31. Wei M, Liu Q, Wang Z, Yang J, Li W, Chen Y, Lu H, Nie J, Liu B, Lv K et al (2020) PuHox52-mediated hierarchical multilayered gene regulatory network promotes adventitious root formation in Populus ussuriensis. New Phytol 228(4):1369–1385
32. Monticellia S, Gentile A, Costantini E, Perazzini M, Damiano C (2011) Agrobacterium-Mediated Transformation in Prunus Species. In: *International Symposium on Genetic Modifications: 2011*

Figures

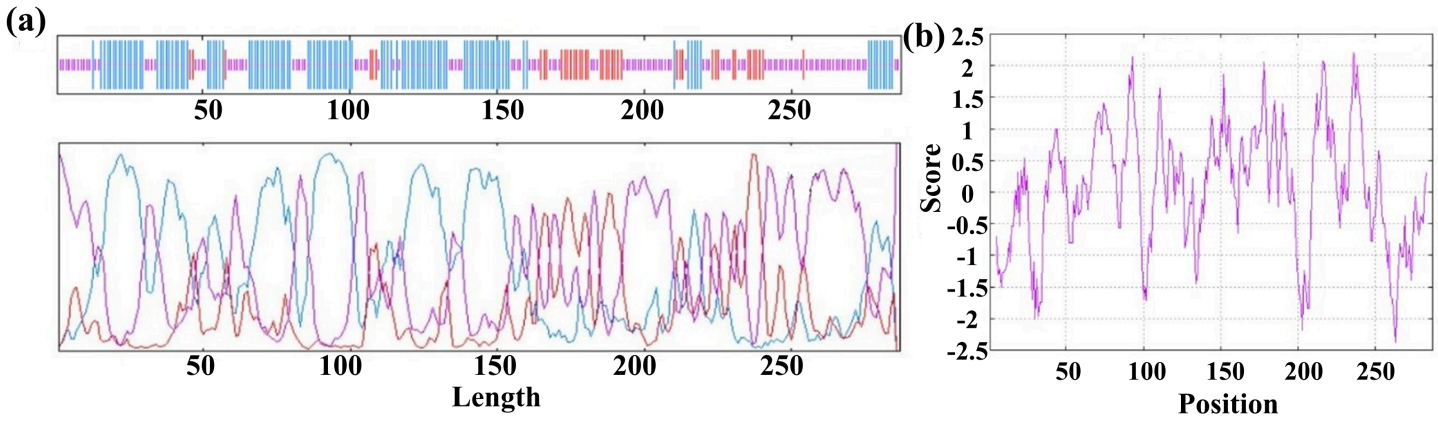


Figure 1

Prediction of secondary structure and hydrophobicity/hydrophilicity of PpSAT4 protein. (a) Secondary structure prediction. Blue, red, and purple represent α-helix, extended strand, and random coil,

respectively. The x-axis indicates the position of amino acid residues. (b) Hydrophobicity/hydrophilicity analysis. The x-axis indicates the position of amino acid residues, and the y-axis indicates the hydropathicity score; positive values represent hydrophobic regions, while negative values represent hydrophilic regions.

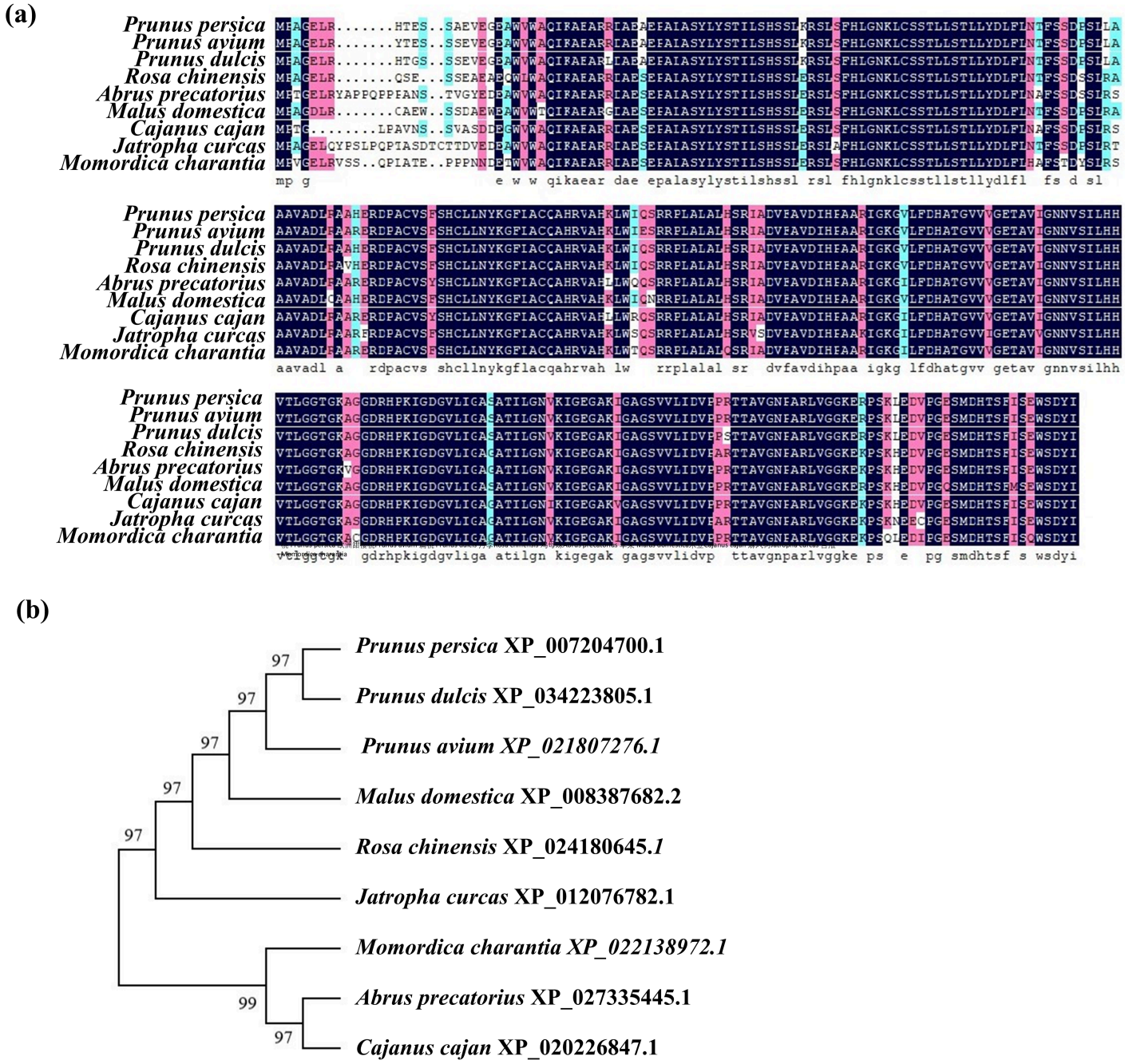


Figure 2

Homology alignment and phylogenetic analysis of the PpSAT4 protein. (a) Multiple sequence alignment of PpSAT4 with SAT4 proteins from different plant species. Dark blue shading indicates highly conserved amino acid residues, while light blue and pink shading represent partially conserved or species-specific residues. (b) Phylogenetic tree of SAT4 proteins constructed using the neighbor-joining method. Numbers at nodes indicate bootstrap values based on 1000 replicates.

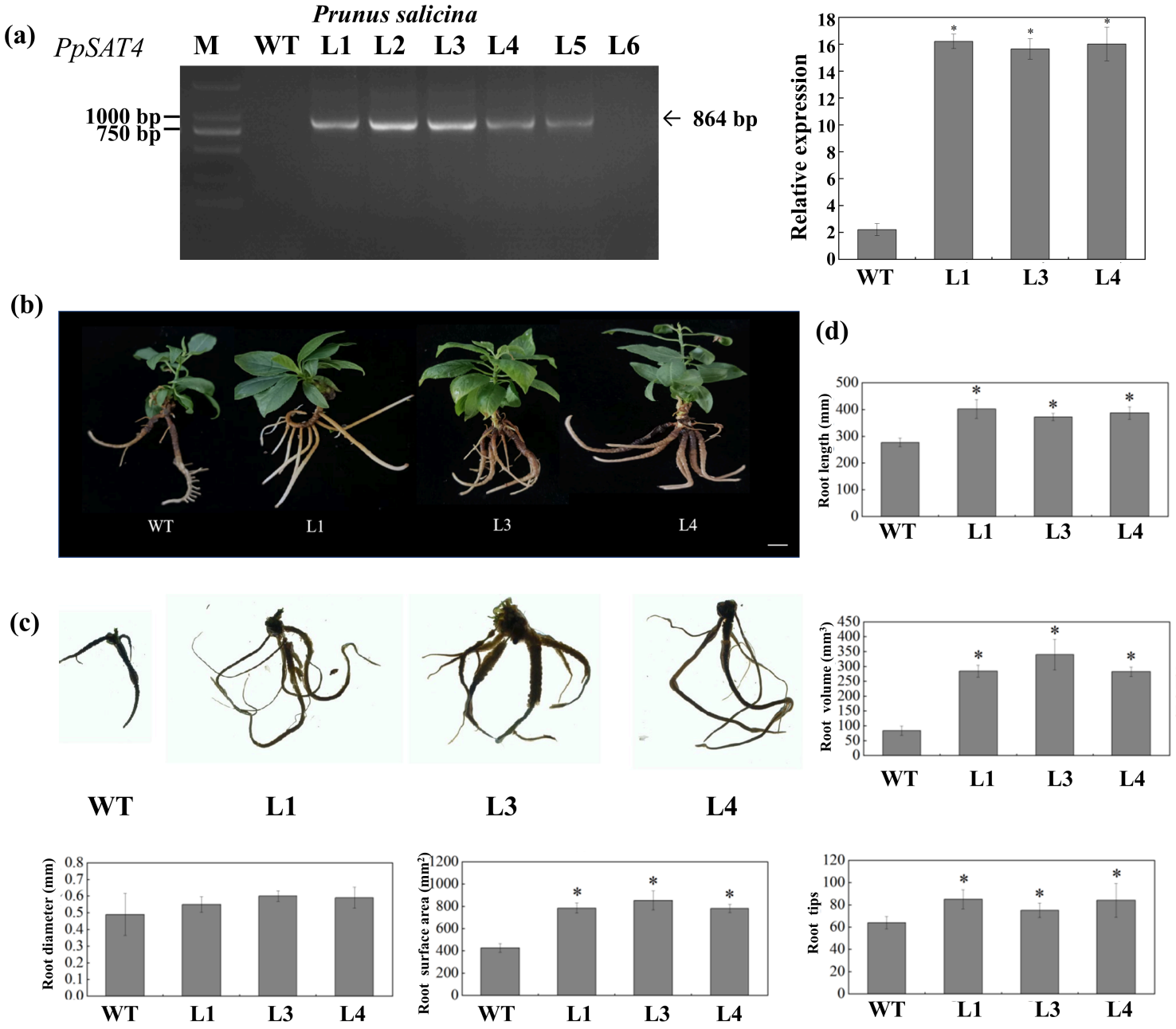


Figure 3

Overexpression of *PpSAT4*promotes adventitious root formation in *Prunus salicina*. (a) PCR identification and expression analysis of *PpSAT4* in transgenic plants. M: DNA marker; WT: wild type; L1-L6: transgenic lines. The arrow indicates the target fragment (864 bp). The histogram on the right shows the relative expression level of *PpSAT4* detected by qRT-PCR. (b) Overall phenotypes of wild-type and overexpression lines. Bar=1 cm. (c) Root phenotypes of wild-type and overexpression lines. (d) Statistical analysis of root morphological parameters, including total root length, root volume, root diameter, root surface area and root tip number. Data are presented as mean ± standard error. * indicates significant difference between transgenic lines and wild type (P<0.05).

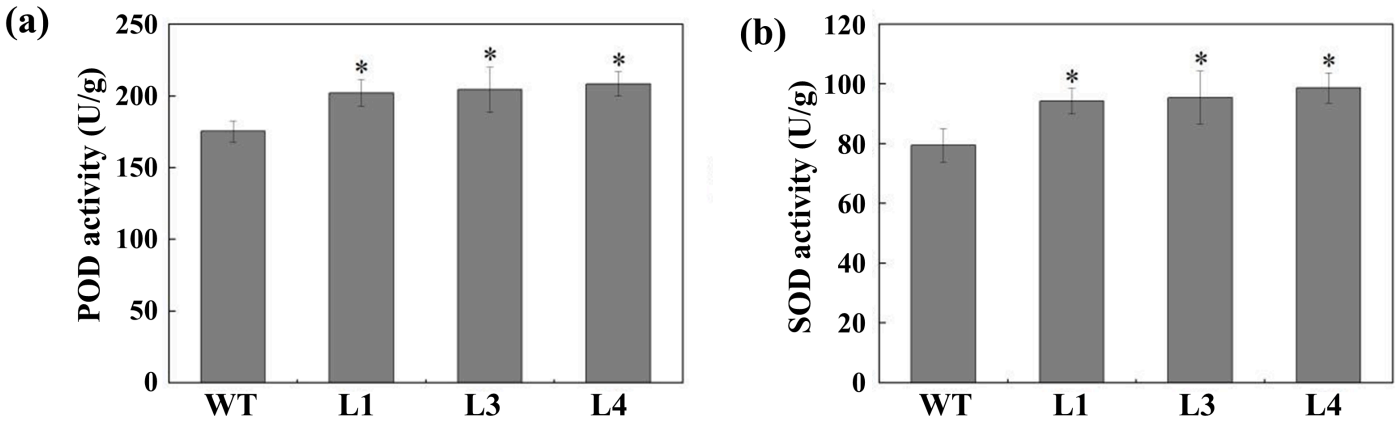


Figure 4

Analysis of antioxidant enzyme activities in *PpSAT4* overexpression lines and Wild-Type plants. (a) Peroxidase (POD) activity; (b) Superoxide dismutase (SOD) activity. WT: wild type; L1, L3, L4: *PpSAT4* overexpression lines. Data are presented as mean ± standard error. * indicates significant difference between transgenic lines and wild type (P<0.05).

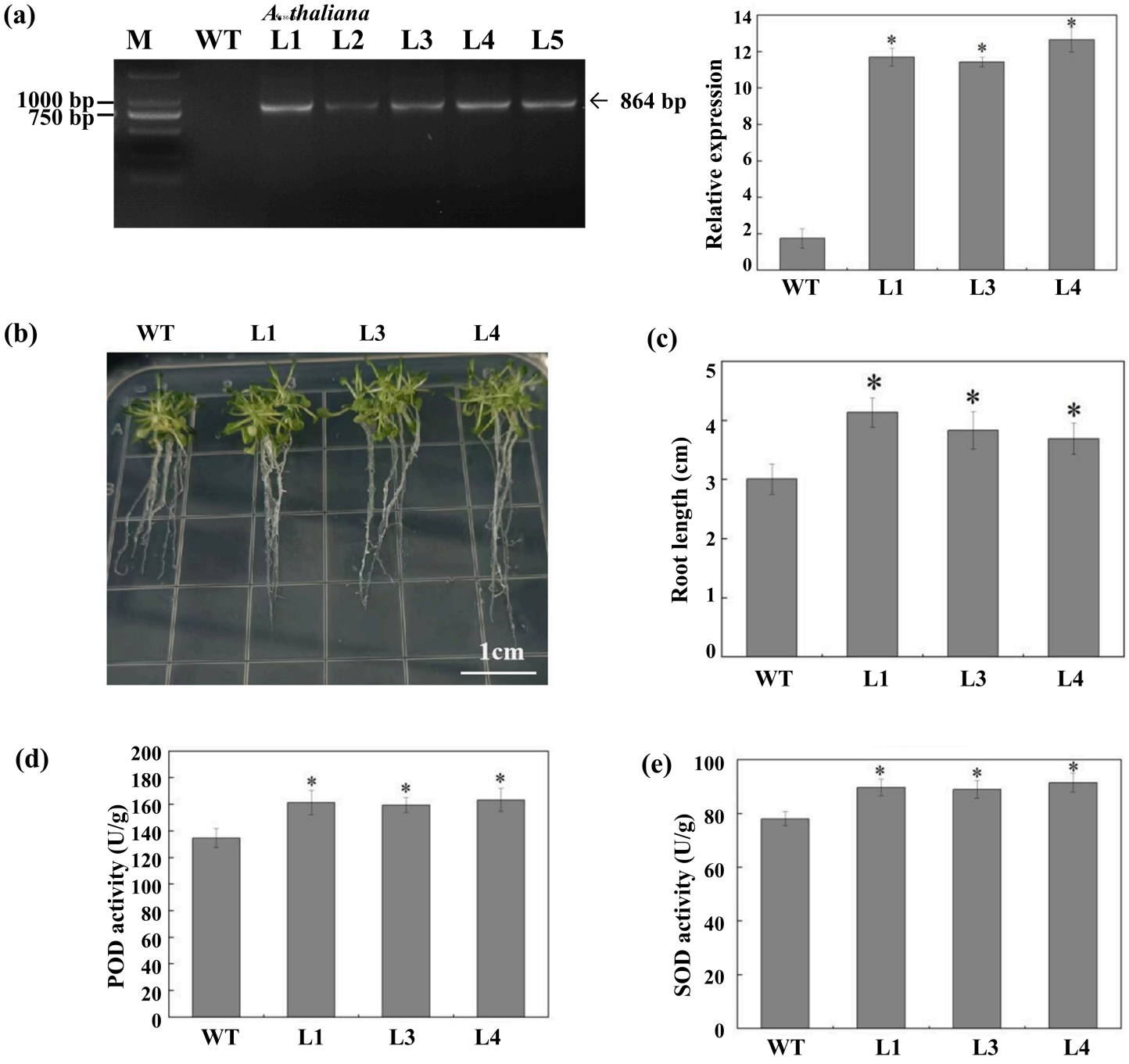


Figure 5

Molecular identification, phenotype and physiological analysis of *PpSAT4*-overexpressing *Arabidopsis* (a) PCR identification and expression analysis of *PpSAT4*in transgenic *Arabidopsis*. Left: PCR identification; M: DNA marker; WT: wild type; L1–L5: transgenic lines. The arrow indicates the target fragment (864 bp). Right: relative expression level of *PpSAT4* detected by qRT-PCR. (b) Seedling phenotypes of WT and overexpression lines (L1, L3, L4). Bar=1 cm. (c) Statistical analysis of primary root length. (d) Peroxidase (POD) activity. (e) Superoxide dismutase (SOD) activity. Data are presented as mean ± standard error. * indicates significant difference between transgenic lines and wild type (P<0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.Primersequence.xlsx