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# Tolerance of transgenic *Arabidopsis thaliana* overexpressing apple *MdAGO4.1* gene to drought and salt stress

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## Summary

The regulatory role of apple *MdAGO4.1* gene in plant drought and salt resistance is unclear. In this study, transgenic *A. thaliana* in which the apple *MdAGO4.1* gene was over-expressed was used to analyze the regulatory effects of the *MdAGO4.1* gene on plant drought and salt resistance, to verify the function of the apple *MdAGO4.1* gene. The seed germination rate, seedling fresh weight and root length of transgenic *Arabidopsis* strains in MS medium containing different concentrations of NaCl and mannitol were better than those of the wild type. The transgenic *A. thaliana* seedlings were more resistant to drought than wild type under drought stress. The transgenic strains were less affected by salt stress than the wild type. Exposure to drought and salt stress reduced the relative elektrolyte leakage, malondialdehyde (MDA), superoxide anion ($O^{2-}$), and hydrogen peroxide ($H_2O_2$) levels of the transgenic strain significantly compared with the levels in the wild type. The levels of proline, protective enzyme activities, and the expression of genes related to drought and salt stress resistance were significantly higher than those of the wild type. These results indicate that *MdAGO4.1* overexpression improved drought and salt tolerance in transgenic Arabidopsis. This study can provide a theoretical basis for future research on stress tolerance mechanisms and breeding new varieties of fruit trees resistant to drought and salt.

**Keyword:** apple, *MdAGO4.1* gene, drought stress, salt stress

## Introduction

Apple (*Malus domestica*) is one of the most widely cultivated fruit trees in the world (Chen et al., 2019). China is the world's top producer of apples, ranking first in both imports and exports (Wu and Pan, 2021). With the rapid development of the apple industry, abiotic stressors, such as drought and salt stress, have become the major limiting factors of plant growth and distribution (Wu et al., 2014). Therefore, the discovery of resistance-related genes provides an important theoretical basis for improving apple resistance and apple fruit quality, which is of great significance in the development of the apple industry in China.

Argonaute (AGO) protein is the core protein in the RNA-induced silencing complex (RISC), which binds to miRNA to block mRNA degradation or translation (Liu et al., 2018). This protein is a key component of the miRNA pathway, consisting of multiple conserved domains (Rivas et al., 2005). *AGO* gene mediates RNA degradation at transcriptional, post-transcriptional, and chromatin levels, which ensures the stability of eukaryotic genomes (Carbonell and Carrington, 2015). AGO proteins are involved in plant growth and development. Overexpression of *OsAGO7* can induce upward curling of rice leaves and enhance leaf erectness (Shi et al., 2007). The catalytic activity of AGO proteins is essential for the early development of embryo (Gerson-Gurwitz et al., 2016). AGO proteins are also involved in plant abiotic stress, such salt stress in rice. The overexpression of *P68* and *AGO1* induces RNA metabolic pathways and effectively eliminates reactive oxygen species (ROS), resulting in tolerance to salt stress (Banu et al., 2015). *OsAGO2* is specifically upregulated in response to cold, salt and drought (Kapoor et al., 2008). Nearly all *CsAGOs* in cucumber responded to these abiotic stresses including cold, high temperature, salt and drought (Gan et al., 2017). Nine *ZmAGO* genes were upregulated under drought and ABA treatments (Zhai et al., 2019).

Over the past few years, the function of plant AGO proteins was reported by several new studies (Fu et al., 2022; Garcia-Ruiz et al., 2015; Hegge et al., 2018). Currently, the regulation and mechanism of AGO protein in woody plants, especially fruit trees exposed to stress is rarely reported. In our previous study, the apple *MdAGO4.1* gene was induced in response to drought, indicating its potential role in resistance to abiotic stress such as drought (Zhou et al., 2016). In this study, the apple *MdAGO4.1* gene was cloned, and its biological function in response to drought and salt stress was analyzed via transgenic technology. The above results provide a basis for further studies investigating the role of abiotic stress resistance genes in plants and lay the foundation for further study of the *AGO* gene response to plant abiotic stress.

## Materials and methods

### Plant materials

The *Arabidopsis thaliana* L. cv. Columbia ('Col') was used for transformation of apple *MdAGO4.1* gene. The wild and transgenic *Arabidopsis thaliana* were used to detect the function of this gene.

### Carriers and strains

Plant overexpression vector: pRI101-EGFP-MYC vector; Gene cloning vector: pMD19-T; Agrobacterium strain: GV3101, *Escherichia coli* strain: DH5α.

### Test methods

*Acquisition of transgenic Arabidopsis lines over-expressing MdAGO4.1*

The cDNA of rootstock leaf of 'ZC9-3' apple, a superior rootstock line developed by the apple rootstock innovation team of Hebei Agricultural University, was used as a template to clone the *MdAGO4.1* gene. The plant overexpression vector pRI101-*MdAGO4.1* was constructed, and the recombinant plasmid was transformed into *Rhizobium radiobacter (*previously *Agrobacterium tumefaciens)* GV3101. The positive strain was detected by PCR. Wild-type *A. thaliana* was infected via flower dipping method. Seeds were screened with MS medium (4.43 g/L MS + 7.8 g/L agar + 30g/L sucrose) containing 50 $mg \cdot L^{-1}$ kanamycin until T3 generation seeds were obtained. The transgenic Arabidopsis lines were detected by PCR and qRT-PCR.

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*Arabidopsis seed germination test*

The seeds of three transgenic strains and wild-type *A. thaliana* were selected and treated with 70% alcohol for 1 min, followed by 2.5% sodium hypochlorite solution for 8 min, during which the tubes were centrifuged upside down several times and rinsed in sterile water 4-5 times before evenly spread on MS medium (Control, without salt or mannitol), MS medium containing 100 or 150 mmol/L NaCl, and 200 or 300 mmol/L mannitol, respectively. The seeds on the culture were vernalized at 4 °C for 3 d and then transferred to culture room (22 °C, 16 h light/8 h darkness). Four days later, the germination rate under each treatment was counted and the seedlings were photographed. Each treatment was replicated three times, with 50 seeds per replicate.

*Osmotic stress and salt stress treatments of Arabidopsis seedlings*

The three transgenic strains and wild-type Arabidopsis seeds were sterilized and evenly sown on MS medium. After vernalization at 4 °C for 3 d, the seedlings were transferred to a culture room (22 °C, 16 h light/8 h darkness). After 5 d of growth, the seedlings were transferred to MS medium (Control, without salt or mannitol), MS medium containing 100 or 150 mmol/L NaCl, 200 or 300 mmol/L mannitol, respectively, in a sterile ultra clean bench. After 7 d of growth in the culture room, the seedlings were photographed to record their growth status. The length of the primary roots and the fresh weight of the seedlings were measured. Each treatment was replicated three times, with 25 seedlings per replicate.

*Drought and salt treatment of adult Arabidopsis seedlings*

Seeds of the transgenic strain and wild type Arabidopsis were sterilized and vernalized at 4 °C for 3 d. Seeds were then sown into nutrient bowls containing substrate (nutrient soil: vermiculite = 1:1), with each nutrient bowl containing ten seedlings. After 25 days of growth, the seedlings were used for drought and salt treatments.

Drought treatment: Full irrigation was conducted before treatment, followed by cessation of watering in the drought treatment group and supply of normal water to the control group. The treatment procedures were photographed and recorded after 15 d. The leaves were sampled, snap-frozen in liquid nitrogen, and transferred to a -80 °C refrigerator for subsequent determination of physiological index (1.3.5 section) and gene expression analysis (1.3.6 section). The drought-treated seedlings were re-watered, photographed and sampled after two days of rewatering. Each treatment was replicated three times, and five nutrient bowls were used each time.

Salt treatment: The nutrient pots were played in cavity trays, with 20 nutrient pots per cavity tray. The control group was cultured normally. In the salt treatment group, 1 L of 300 mmol/L NaCl solution was poured into the cavity trays every 1 day, photographed and recorded after 8 d. The leaves were sampled, snap-frozen in liquid ‘nitrogen, and transferred to a -80 °C refrigerator for subsequent determination of physiological index (1.3.5 section) and gene expression analysis (1.3.6 section). Each treatment was replicated three times, and five nutrient bowls were used each time.

*Physiological and biochemical indicators*

Superoxide dismutase activity (SOD) was determined by nitrogen blue tetrazolium (NBT) method (Sharma et al., 2020); catalase activity (CAT) was determined via UV absorption method (Sharma et al., 2020). Peroxidase (POD) activity was determined using the guaiacol method (Sharma et al., 2020). The MDA content was determined via thiobarbituric acid method (Sharma et al., 2020). The proline content was determined via acid ninhydrin staining (Sharma et al., 2020). The superoxide anion content was determined according to the method of Zhang (Zhang et al., 2007). The relative conductivity was determined as described by Yang (Yang et al., 2020b). The $H_2O_2$ content was determined using a kit ordered from Suzhou GRS Biotechnology Co., Ltd.

*Gene expression analysis*

We examined the effect of overexpression of apple *MdAGO4.1* gene on the expression of reported drought or cold tolerance genes, to investigate the mechanism by which this gene regulates drought or salt tolerance in plants. According to previous reports, we selected nine drought-related genes (*AtRD29A, AtRD29B, AtRD22, AtERD15, AtERD11, AtSnRK2.4, AtDREB1A, AtDHAR, AtDHAR2*) and nine salt resistance-related genes (*AtNHX1, AtNHX2, AtNHX3, AtP5CS2, AtSOS1, AtSOS2, AtSOS3, AtDHAR, AtDHAR2*) to detect their expression level.

Total RNA of leaf was extracted from wild-type and transgenic Arabidopsis plants under different treatments using a polymeric RNA isolation kit (M5 Plant RNeasy Complex Mini Kit, Beijing, China). The cDNA was obtained by reverse transcription, and gene-specific primers were designed (Tab. 1).

**Tab. 1:** The primers for qRT-PCR

| Gene name | Primer-Sense | Primer-Antisense |
|---|---|---|
| *AtRD29A* | TTCTGTAAGGACGACGTTTACA | CGTACTCGTTACATCCTCTGTT |
| *AtRD29B* | GAAACCAAAGATGAGTCGACAC | TTTTTCGTAAACCGGAGTCAAC |
| *AtRD22* | GACTTTCGATTTTACCGACGAG | CGCTACCGGTTTTACCTTTATG |
| *AtERD15* | TTCGACTTGGTACCCTGATTAC | TTCGACTTGGTACCCTGATTAC |
| *AtERD11* | CCCCTTTGGTAAAGTTCC | CCCCTTTGGTAAAGTTCC |
| *AtSnRK2.4* | GAGGAAATGGGGATGCAGAT | TTCTCACTTCTCCACTTGCG |
| *AtDREB1A* | AGGAGACGTTGGTGGAGGCT | ACGTCGTCATCATCGCCGTC |
| *AtDHAR* | GTATCCTGATCCACCTCTCAAG | GCTTTGGTGCTAAGCTAAGATC |
| *AtDHAR2* | AGGCTTTGGTTGATGAGTTAGA | TGGTAAAGCTTTGGTGCTAAAC |
| *AtNHX1* | AGCCTTCAGGGAACCACAAT | CTCCAAAGACGGGTCGCATG |
| *AtNHX2* | ATCACTGCTTTATTGATTGGGC | GCGCCAAAAGCCATAATAGTTA |
| *AtNHX3* | GTGACGAATAAACTAGCAGCTG | CCATCGATTCTCTTCAAGCAAG |
| *AtP5CS2* | GGGACAAGTTGTGGATGGAGAC | TGGTACAAACCTCAAGGAACAC |
| *AtSOS1* | ATTTTGATGCAGTCAGTGGATG | GCAAGCAGATTCTAGTCTTTCG |
| *AtSOS2* | GCGAACTCAATGGGTTTTAAGT | CTTACGTCTACCATGAAAAGCG |
| *AtSOS3* | CCGGTCCATGAAAAAGTCAAAT | CTCTTTCAATTCTTCTCGCTCG |
| *AtPOD* | GGTTGTGACGCATCCATCTTGTT | TCCACCGCAGCTTTCATTCTAT |
| *AtSOD2* | GGTGACCTGGGAAACATAAATG | CCAGTCAGAGGAATCTGATTGT |
| *AtCAT3* | TCACAGCCACGCCACTAA | AGAACCAAGCGACCAACC |
| *AtActin* | GGAAAGTCCTACCAGCATTG | ATCTATTGTCTCCCACGAAG |
| *MdAGO4*.1 | ACCACTCGGAGTTAGGTG | TTTGGACGCCGTAATCT |

**Statistical analysis**

Microsoft office Excel was used to process the data. IBM SPSS Statistics 23.0 software was used for statistical analysis. Duncan’s multiple comparison was performed using one-way ANOVA ($p < 0.05$).

## Results

**Identification of transgenic Arabidopsis overexpressing *MdAGO4.1***

*A. thaliana* was infected via inflorescence infection. Matured seeds were disinfected and spread on MS medium containing 50 $mg \cdot L^{-1}$ kanamycin to screen out green and normal resistant seedlings. DNA was extracted from grown resistant seedlings the transgenic Arabidopsis lines were detected via PCR using specific primers (F: TGGGTGCAGAGGATTCCATT; R: CGTTGCAAGGACACCAAAGA) with the plasmid expression vector as the positive control (Fig. 1a). The total RNA of transgenic *A. thaliana* was extracted, and the cDNA was synthesized. The specific primers of target gene (Tab. 1) were used to detect the expression level by qRT-PCR (Fig. 1b). The results showed that the selected

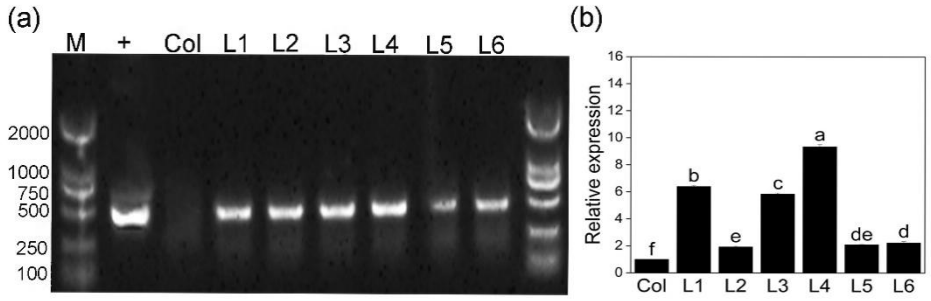


**Fig. 1:** Identification of transgenic *Arabidopsis thaliana* overexpressing *MdAGO4.1*. (a) Identification of *MdAGO4.1* transgenic lines by PCR. MDNA marker DL2000; +, positive control; (b) Identification of *MdAGO4.1* transgenic *A. thaliana* lines by qRT-PCR.

transgenic Arabidopsis lines were all positive strains. Due to the low transcriptional expression of L2, L5 and L6 strains, L1, L3 and L4 strains were selected.

### Analysis of seed germination rate of *MdAGO4.1* transgenic *A. thaliana* under osmotic and salt stress

After 4 days of treatment, almost all the seeds of wild type and transgenic *A. thaliana* lines overexpressing *MdAGO4.1* in MS medium germinated. In the presence of MS medium containing NaCl and mannitol, the seed germination rates of wild type and transgenic *A. thaliana* lines overexpressing *MdAGO4.1* decreased significantly, while the seed germination rate of transgenic Arabidopsis was significantly higher than that of wild type (Fig. 2a-c).

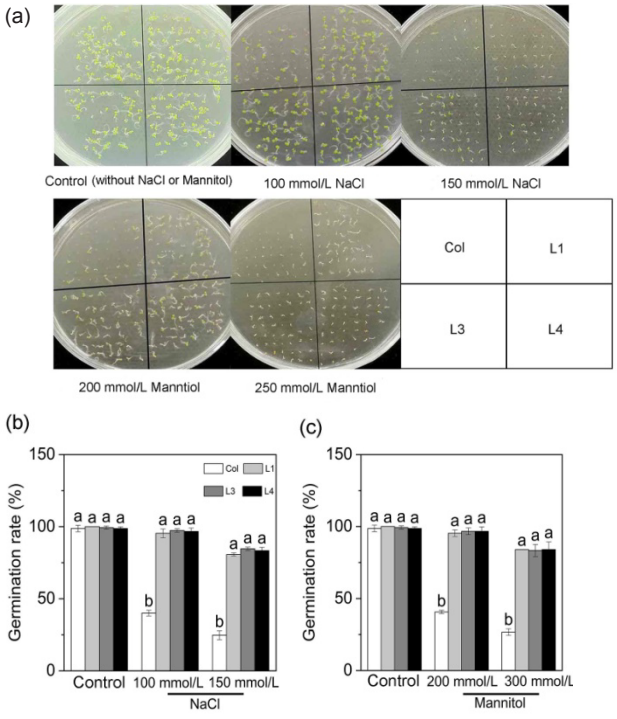


**Fig. 2:** Seed germination rates of transgenic and wild-type Arabidopsis under NaCl and mannitol treatment: (a) Seed germination phenotype of transgenic and wild-type *A. thaliana* lines under NaCl and mannitol treatments; (b) Seed germination rate under sodium chloride treatment; (c) Seed germination rate under mannitol treatment.

### Phenotypic analysis of *MdAGO4.1* transgenic *A. thaliana* seedlings under osmotic and salt stress

In MS medium, the growth vigor of wild-type seedlings and *MdAGO4.1*-overexpressing transgenic Arabidopsis lines was similar. In the presence of 100 mmol/L or 150 mmol/L NaCl, and 200 mmol/L or 300 mmol/L mannitol, *MdAGO4.1*-overexpressing transgenic *A. thaliana* lines grew better than wild type (Fig. 3a). In the presence of 100 mmol/L or 150 mmol/L NaCl, and 200 mmol/L or 300 mmol/L mannitol, the main root length and fresh weight of *MdAGO4.1* transgenic Arabidopsis lines were greater than in wild type (Fig. 3b-e).

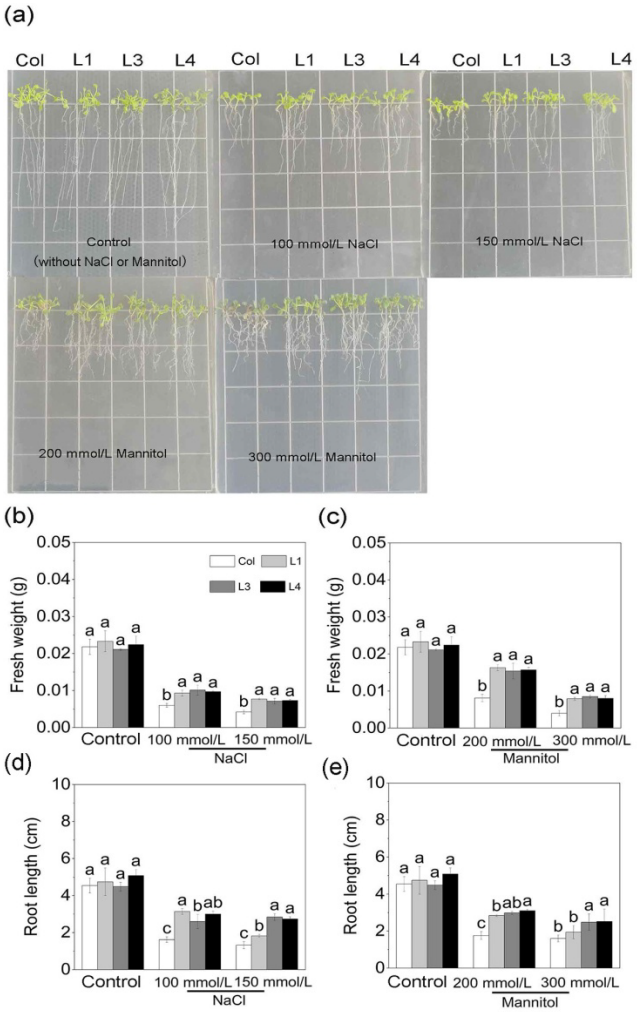


**Fig. 3:** Phenotypic analysis of seedlings of transgenic Arabidopsis lines and wild type under NaCl and mannitol treatment: (a) Phenotypes of transgenic lines and wild-type seedlings under NaCl and mannitol treatment; (b-c) Fresh weight of transgenic lines and wild-type seedlings under NaCl and mannitol treatment; (d-e) Length of the main root of transgenic lines and wild-type seedlings under NaCl and mannitol treatment.

**Resistance analysis of *MdAGO4.1* transgenic *A. thaliana* under drought and salt stress**

Both wild-type and transgenic Arabidopsis showed water deficiency symptoms after 15 days of drought treatment. However, compared with wild type, the transgenic lines showed significantly lighter symptoms. After 2 days of re-watering treatment, the wild-type basically died, while the transgenic lines mostly recovered. After 8 days of salt treatment, the growth of all transgenic and wild-type lines was inhibited, but the wild-type lines were affected significantly more by salt stress than transgenic lines (Fig. 4).

Under drought and salt stress, the MDA content and relative conductivity of the transgenic *A. thaliana* lines were significantly lower than those of the wild type, while the proline content was significantly higher than in wild type, indicating that the transgenic lines were damaged less by drought and salt stress (Fig. 5a-c).

Under normal growth condition, the levels of superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$) in transgenic lines were not significantly different from the levels in wild type. Under drought and salt stress, the $O_2^-$ and $H_2O_2$ levels of transgenic Arabidopsis lines were lower than in wild type (Fig. 6a-b). Under normal growth conditions, no significant differences in POD, SOD and CAT activities were detected between transgenic lines and wild type. Under drought and salt stress, the activities of POD, SOD and CAT in transgenic lines were significantly higher than in wild type (Fig. 6c-e). The analysis of antioxidant enzyme-related genes showed that the expression of *AtPOD, AtSOD2,* and *AtCAT3* genes in transgenic *A. thaliana* lines was higher than in wild type under drought and salt stress (Fig. 7).

**Effects of drought and salt stress on the expression of drought- and salt-related genes in *A. thaliana***

Under normal conditions, there was no significant difference in the expression of drought-related genes (*AtRD29A, AtRD29B, AtRD22, AtERD15, AtERD11, AtSnRK2.4, AtDREB1A, AtDHAR, AtDHAR2*) between transgenic *A. thaliana* lines and wild type. Under drought stress, the expression of drought-related genes in transgenic *A. thaliana* lines was significantly higher than in wild type (Fig. 8). Under salt stress, the expression of salt resistance-related genes (*AtNHX1, AtNHX2, AtNHX3, AtP5CS2, AtSOS1, AtSOS2, AtSOS3, AtDHAR, AtDHAR2*) in transgenic lines was significantly higher than in wild type (Fig. 9).

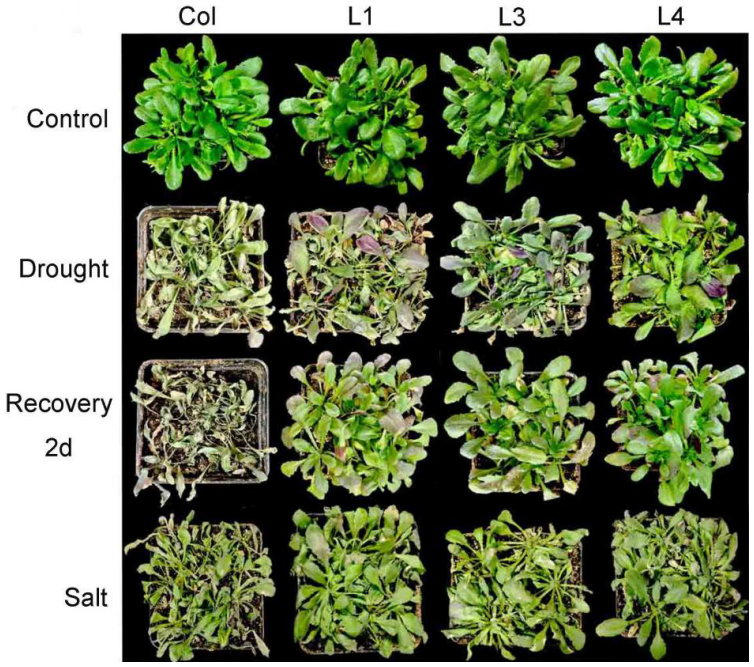


**Fig. 4:** Phenotypes of transgenic and wild-type *A. thaliana* lines under drought and salt treatments

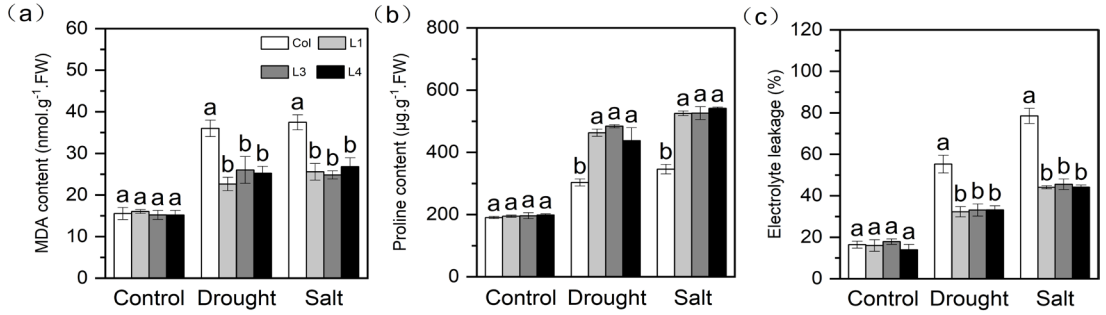


**Fig. 5:** Analysis of related physiological indicators of transgenic and wild-type Arabidopsis seedlings under osmotic and salt stress. (a) MDA content of transgenic and wild-type Arabidopsis seedlings under osmotic and salt stress. (b) Proline content of transgenic and wild-type Arabidopsis seedlings under osmotic and salt stress. (c) Relative conductivity of transgenic and wild-type *A. thaliana* seedlings under osmotic and salt stress.

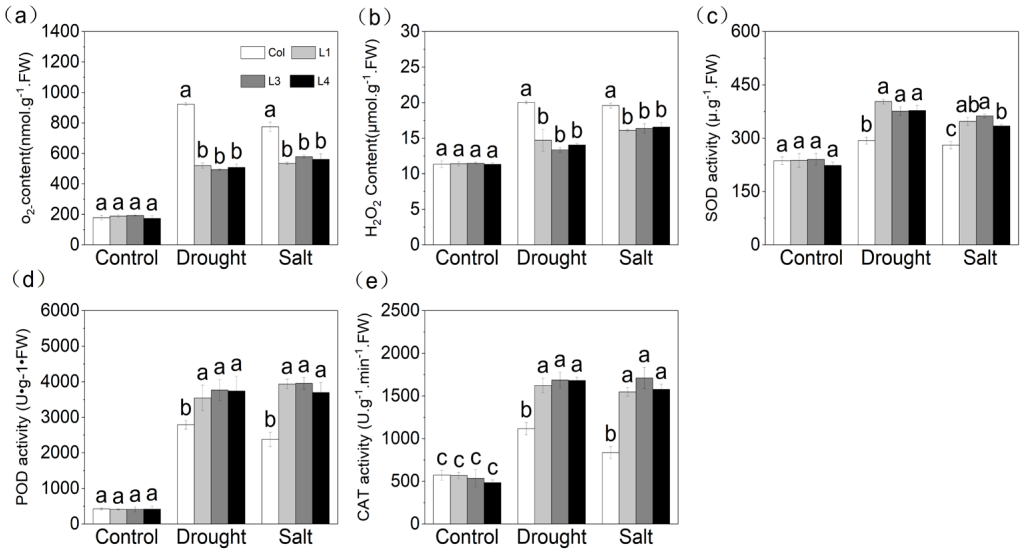


**Fig. 6:** Analysis of reactive oxygen species (ROS) and antioxidant enzyme activities in transgenic and wild-type *A. thaliana* under drought and salt treatments. (a) Superoxide anion ($O_2^-$) level in transgenic and wild-type *A. thaliana* under drought and salt treatments. (b) Hydrogen peroxide ($H_2O_2$) level in transgenic and wild-type *A. thaliana* under drought and salt treatments. (c) SOD activity in transgenic and wild-type *A. thaliana* under drought and salt treatments. (d) POD activity in transgenic and wild-type *A. thaliana* under drought and salt treatments. (e) CAT activity in transgenic and wild-type Arabidopsis under drought and salt treatments.

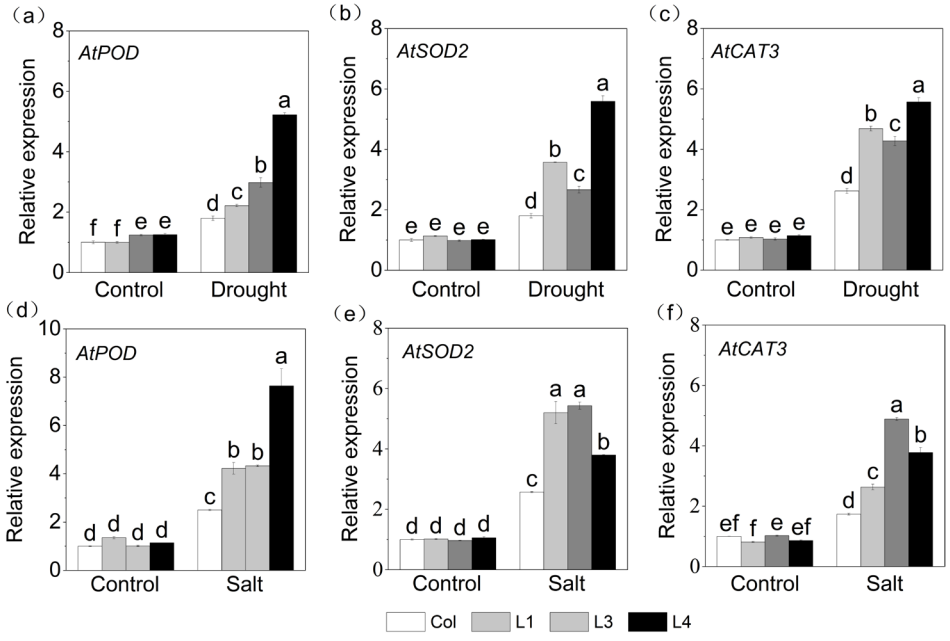


**Fig. 7:** Expression of antioxidant enzyme-related genes in transgenic and wild-type *A. thaliana* lines under drought and salt stress.

## Discussion

Stress affects seed germination. Increased levels of stress inhibit seed germination in direct proportion (Hu et al., 2012; Zhao et al., 2014). Plant growth and development is inhibited by stress. Further, stress alters plant morphology, affecting physiological and biochemical and metabolic characteristics (Baath et al., 2017). AGO protein plays an important role in plant growth and development, stress resistance and other physiological events (Meister, 2013). Studies have shown that AGO4 may end the dormancy of peanuts and promote seed germination (Chi et al., 2011). This study showed that the seed germination rate, seedling root length and fresh weight of wild type and transgenic lines were significantly decreased by NaCl and mannitol treatment. However, the seed germination rate, seedling root length and fresh weight of transgenic lines were significantly higher than in wild type. The results showed that overexpression of *MdAGO4.1* enhanced the resistance of *A. thaliana* seeds and seedlings to osmotic and salt stress. Drought treatment induced wilting in wild-type lines and significant loss of water in the leaves, while the transgenic lines showed less wilting. After 2 days of rewatering, the wild-type lines almost died, while most of the transgenic lines recovered. Salt treatment inhibited the growth of all lines, but the transgenic lines were less stressed. The above results indicate that *MdAGO4.1* was involved in the defense response of apple to drought and salt stress,

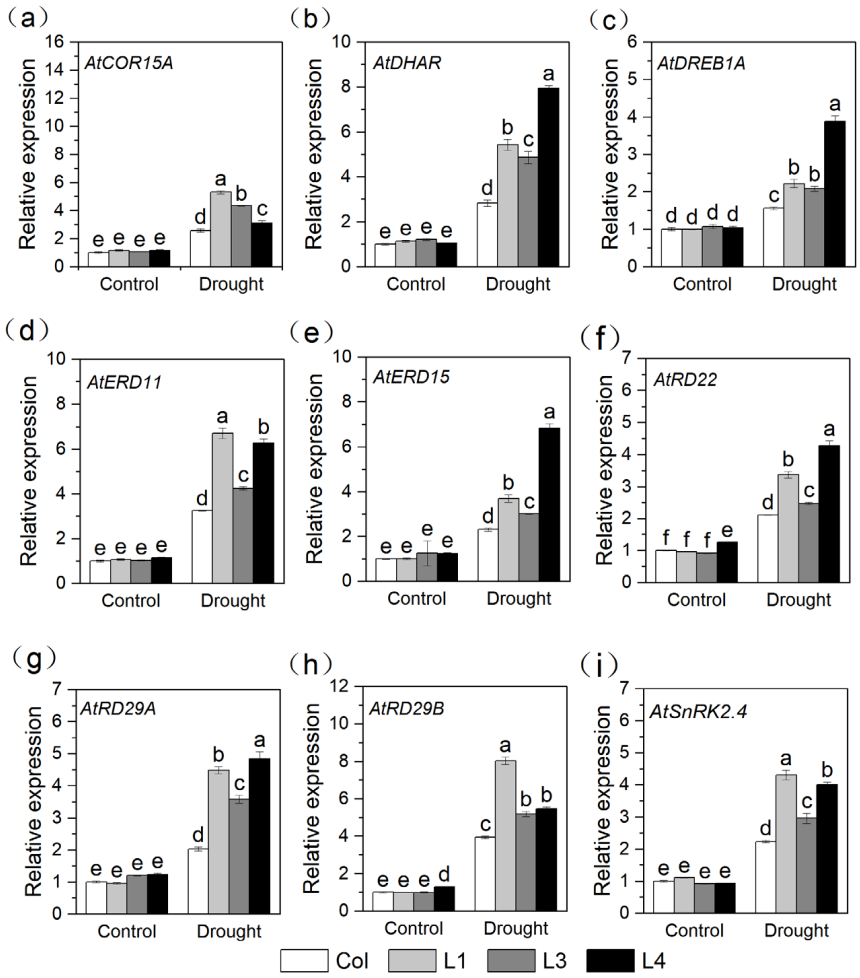


**Fig. 8:** Expression of drought resistance-related genes in transgenic and wild-type *A. thaliana* under drought stress.

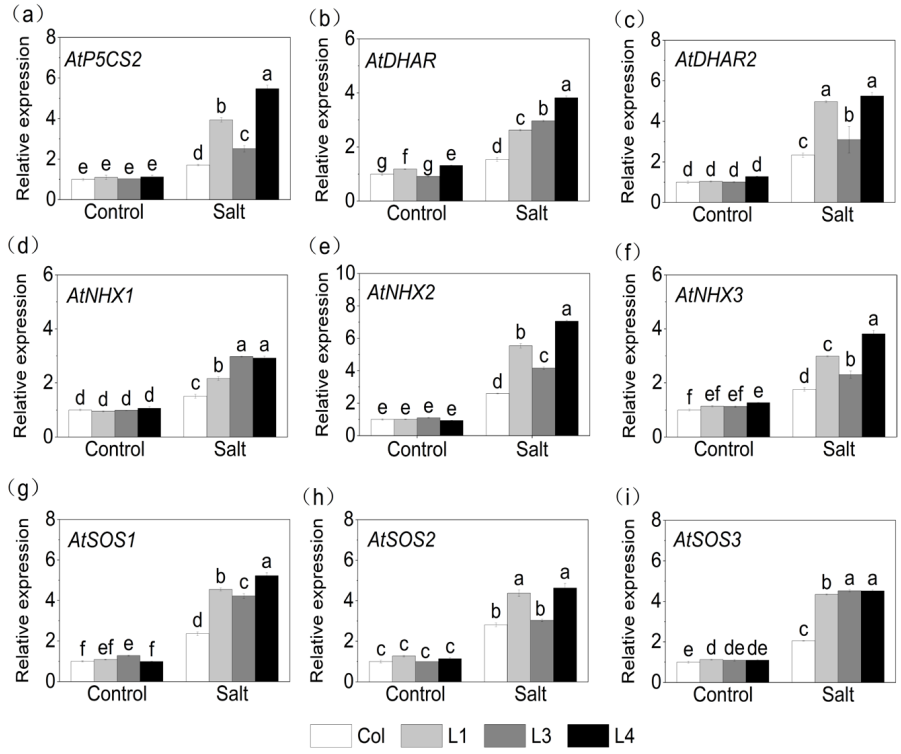


**Fig. 9:** Expression of salt tolerance-related genes in transgenic and wild-type *A. thaliana* under normal and salt stress conditions.

and overexpression of *MdAGO4.1* gene improved the drought and salt resistance of plants.

Relative conductivity and MDA content reflect the extent of stress-induced damage in plant cells (Trovato et al., 2008). Stress induces the formation and accumulation of ROS resulting in cell damage (Ullah et al., 2018). Proline is one of the important compounds associated with osmotic regulation in plants and regulates the osmotic potential during the plant growth and development (Movahedi et al., 2012; Song et al., 2019). This study showed that the relative conductivity and MDA content of transgenic lines were significantly lower than in wild type under drought and salt stress, while the proline content was significantly higher than that of wild type. Overexpression of *MdAGO4.1* contributes to cellular homeostasis and alleviates the degree of cell damage under stress. The levels of these physiological indicators were consistent with the phenotypes of transgenic *A. thaliana* lines under drought and salt stress, indicating that overexpression of *MdAGO4.1* transgenic *A. thaliana* enhanced resistance to drought and salt stress.

Plants are induced by abiotic stress, and excessive ROS levels in leaves trigger membrane lipid peroxidation and destruction of membrane integrity (Parihar et al., 2015; Yang and Guo, 2018). Plants enhance their cellular detoxification capacity via antioxidant enzymes such as CAT, SOD, and POD to defend against ROS damage (Chowhan et al., 2014; Das and Roychoudhury, 2014). Overexpression of hexokinase 1 (*OsHXK1*) led to excessive ROS accumulation in rice with downregulated *OsAGO2* expression (Zheng et al., 2019). The results of this study showed that the levels of $H_2O_2$ and superoxide anion in leaves of wild-type and transgenic *A. thaliana* strains increased under drought and salt stress compared with normal condition. However, their levels were significantly higher in the wild type than in transgenic strain. The POD, SOD and CAT activities of the *MdAGO4.1*-overexpressing *A. thaliana* lines were significantly higher than those of the wild type under drought and salt stress. The overexpression of *MdAGO4.1*-transgenic *A. thaliana* enhanced antioxidant enzyme activity and reduced the structural damage in cells due to ROS, which in turn improved the resistance of plants to drought and salt stress. The expression of antioxidant enzyme-related genes (*AtPOD, AtCAT3, AtSOD2*) in the transgenic *A. thaliana* strain overexpressing *MdAGO4.1* was significantly higher than in the wild type. Therefore, *MdAGO4.1* overexpression in *A. thaliana* affects the transcription of antioxidant enzyme genes, resulting in higher antioxidant enzyme activities following exposure to drought and salt stress. The ROS accumulated in the plant are scavenged, thereby mitigating the damage.

DHAR (Dehydroascorbate reductase)gene plays an important role in plant response to external environmental stress, and its overexpression in tobacco enhanced resistance to low temperature, salt stress, drought, and other adversities (Eltayeb et al., 2006). The plant *SnRK* family includes *SnRK1*, *SnRK2* and *SnRK3*. *SnRK2* is associated with a variety of abiotic stress response pathways, such as drought, salt, ABA and other stress treatments (Lin et al., 2021). *DREB* (Dehydration responsive element binding protein) is involved in a variety of responses to adversities in plants, including abiotic stressors such as low temperature, drought, high temperature, and high salt concentrations (Chen et al., 2022; Donde et al., 2019; Liang et al., 2017; Sakuma et al., 2006). Overexpression of rice *OsDREB1A* enhanced salt tolerance and drought resistance in *A. thaliana* (Dubouzet et al., 2003). The *DREB* gene significantly improved the resistance of wheat to drought and adversity (Yang et al., 2020a). *ERD* (Early responsive to dehydration)is rapidly induced by stress and is involved in toxic stress response and tolerance (Nakashima et al., 1997). The expression of the *ERD15* gene in tomato was increased in response to drought, salt stress, and low temperature (Ziaf et al., 2011). *RD29* genes, including *RD29A* and *RD29B*, were induced by drought, cold, and high salt stresses, and these genes encode hydrophilic proteins that induce stress tolerance in plants (Jia et al., 2012). RD22-like proteins play an important role in plant response and resistance to a variety of abiotic stressors. This study showed that the expression of drought-related genes (*AtRD29A, AtRD29B, AtRD22, AtERD15, AtERD11, AtSnRK2.4, AtDREB1A, AtDHAR, AtDHAR2*) was significantly higher in the transgenic strain than in the wild type. Therefore, it is hypothesized that *MdAGO4.1* overexpression affects the expression of stress-related genes and thereby improves the drought tolerance of the transgenic strain.

Previous studies have shown that salt stress leads to imbalance in osmoregulation and ion toxicity in plants. The overexpression of *SOS1*, a gene coding for $Na^+/H^+$ reverse transporter protein on the cell membrane of *A. thaliana*, and *NHX*, a gene coding for $Na^+/H^+$ reverse transporter protein on the vesicle membrane, enhances salt resistance in plants (Huazhong et al., 2002). SOS2 and SOS3 regulate the expression of *SOS1* and also respond to salt stress (Ghosh et al., 2022). Under stress conditions, proline content can be increased by increasing the expression of *P5CS* gene (Verma et al., 2020). Reduced expression of the *AtP5CS1* gene in *A. thaliana* decreased the accumulation of proline to the extent that salt tolerance was reduced (Chen et al., 2013). The expression of salt resistance-related genes (*AtNHX1, AtNHX2, AtP5CS2, AtHSP17.8, AtSOS1, AtSOS2, AtSOS3, AtDHAR, AtDHAR2*) in *MdAGO4.1*-overexpressing *A. thaliana* was significantly higher than in wild type under salt stress. Therefore, it is speculated that the overexpression of *MdAGO4.1* enhances the salt tolerance of transgenic lines by affecting the expression of the above salt stress-related genes.

## Conclusion

Under different concentrations of mannitol and NaCl treatment, transgenic *A. thaliana* overexpressing *MdAGO4.1*exhibited significant increase in seed germination rate and seedling fresh weight compared with wild type. It also enhanced tolerance to osmotic and salt stress. Under drought and salt stress, the proline content, the activities of SOD, POD and CAT of *MdAGO4.1* transgenic *A. thaliana* were significantly higher than those of wild type. Relative conductivity, MDA content, $O_2^-$ and $H_2O_2$ content were reduced significantly, and the transcription level of stress-induced genes was significantly higher than in wild type, indicating that overexpression of *MdAGO4.1* improved the drought and salt tolerance of transgenic *A. thaliana*.

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**Authors' contributions**

MingxiaoLiu carried out the experiment; Xiaohan Li wrote the paper; Baoying Yin and Ye Sun analyzed the data; Bowen Liang and Zhongyong Li managed the materials; Xueying Zhang provided the material; Jizhong Xu and Shasha Zhou designed the experiment.

**Conflict of interest**

No potential conflict of interest was reported by the authors.

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