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Overexpression of *HaASR1* from a Desert Shrub, *Haloxylon ammodendron*, Improved Salt Tolerance of *Arabidopsis thaliana*

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Abstract: Salt stress causes reduced plant growth and alters the plant development process, resulting in a threat to global crop production. The exploring of unique genes conferring to salt tolerance from plants that inhabit extreme environments remains urgent. *Haloxylon ammodendron* is a desert xero-halophyte shrub with a strong tolerance to drought and salt stresses. We previously reported that the drought tolerance of *Arabidopsis thaliana* was improved by the overexpression of *HaASR1* from *H. ammodendron*. In this work, the effects of *HaASR1* overexpression on the salt tolerance of *Arabidopsis* were investigated. *HaASR1* overexpression significantly enhanced the growth of *Arabidopsis* lines under salinity and plant tissue water content through enhancing the osmotic adjustment ability, maintaining the membrane integrity, improving the chlorophyll content and leaf area, and thereby enhancing photosynthesis capacity. Taken together, the overexpression of *HaASR1* from *H. ammodendron* improved the salt tolerance of the transgenic lines of *Arabidopsis*. These results indicated that *HaASR1* from *H. ammodendron* has potential application values in increasing the salt tolerance of grass and crop plants by genetic engineering.

Keywords: *Haloxylon ammodendron*; *HaASR1*; *Arabidopsis*; salt tolerance

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1. Introduction

Abiotic stresses, such as salinity, drought, heat and coldness, pose severe challenges to plant growth [1]. High soil salinity poses a serious threat to global food security by affecting normal plant growth and crop yield through ion toxicity, osmotic stress, and nutrient deficiency [2,3]. It is estimated that 6–7% of the world's total land area is affected by soil salinity [4]. High salt content is a characteristic of saline soil, and this is mainly affected by sodium chloride (NaCl) [4,5]. A combination of global climate change, over-fertilization, and irrigation-dependent farming practices is increasing the amount of farmland affected by salinity each year, especially in arid and semi-arid regions [6]. In order to adapt to habitat changes, plants have gradually developed morphological, physiological, and molecular response mechanisms, and different plant species differ significantly in their use of these mechanisms [7,8]. In recent years, research on stress response genes of plants living in extreme environments has attracted wide attention. It is very important to explore these genes and verify their functions [9].

ASR (abscisic acid, stress, ripening) is a family of small proteins induced by ABA, maturation, and biological and abiotic stress, belonging to the seventh category of the LEA family [10]. ASR has been found in various plant species, including potato, grape, corn, strawberry, rice, lily, peanut, banana, etc., and the number of ASR varied greatly

among different species. However, no ASR was found in *Arabidopsis thaliana*, which makes it difficult to research the function of ASR [11–13]. Under drought and osmotic stress, ASR expression can slow down water loss, promote the regulation of amino acid synthesis, and enhance the antioxidant capacity of plants [14–16]. Simultaneously, ASR can also reduce intracellular Na^+ content under salt stress, decrease cell damage, maintain osmotic balance, and regulate fruit development and ripening [11,17–19]. An increase in soil salinity causes plants to suffer osmotic stress, the acceleration of water loss in leaves, the inhibition of cell proliferation, and slow plant growth [20,21]. Transgenic lines of *Nicotiana tabacum* overexpressing the *SbASR1* of *Salicornia europaea* had lower Na^+ accumulation than WT [17]. The transfer *MpASR* of *Musa nana* into *Arabidopsis* could improve the germination rate, soluble sugar content, root growth and salt tolerance [14]. The transfer ASR1 of *Oryza sativa* into *Yeast* cells can enhance the expression of antioxidant enzymes and promote the growth under salt stress [22]. At present, many studies reports that ASR can positively regulate plant abiotic stress tolerance [23]. The expression of ASR enhanced plants' tolerance to drought, high salinity and low temperature. The overexpression of *BdASR4* of *Brachypodium* could improve the growth of *Brachypodium distachyon* transgenic lines under drought stress [24]. *ZmASR1* from maize was initially found to enhance maize water use efficiency and dry weight under moderate water shortage conditions [25]. Later study results showed that the overexpression of *ZmASR1* could improve the seed harvesting of transgenic lines under drought stress [11]. Meanwhile, *ZmASR1* has the ability to improve maize tolerance to low temperature stress [26,27]. *OsASR1* also has the function of improving rice adaptability to various stress environments. Transgenic rice lines overexpressing *OsASR1* had stronger resistance to low temperature stress. Overexpressed *OsASR1* in rice made the seedlings of the transgenic lines have stronger tolerance to higher salinity and osmotic stress [28]. In subsequent studies, *OsASR1* was found to regulate the aluminum tolerance of rice [29]. Meanwhile, *OsASR5* positively regulated the tolerance of *Arabidopsis* and rice to osmotic and drought stresses [30]. *OsASR2* can participate in the regulation of rice tolerance to abiotic stresses, including disease resistance and drought tolerance [31]. Transgenic tobacco line expressing *TaASR1* from wheat had stronger resistance to osmosis and drought stress [15]. ASR proteins were found in the cytoplasm, nucleus and chloroplast. ASR proteins in cytoplasm can be activated by stress signals, and some of them cooperate with other osmotic regulators in the form of monomers to protect stress response proteins. In the nucleus, activated ASR protein acts as a transcription factor to activate the expression of downstream stress response genes, at which time it participates in plant cells' response to environmental signals in the form of dimer [14,15,18]. Although there are many reports on the regulation of ASR on abiotic stress tolerance, the specific mechanism of how ASR plays a role in plant stress response remains to be further investigated.

Haloxylon ammodendron is a xerohalophytic shrub in the chenopodiaceae family [32]. This species has a distinguished tolerance to environmental stresses and can survive even when soil water content drops to 1.0%. It is widely distributed in the arid desert regions of Asia [33–35]. It has been reported that large amounts of Na^+ were absorbed by the roots of *H. ammodendron* and transferred to photosynthetic tissues for osmoregulation [36]. Meanwhile, Na^+ can be applied to accelerate the growth of *H. ammodendron* and enhance drought tolerance [37]. The stem and fruit of *H. ammodendron* are rich in nutrients, and the parasitic *Cistanche deserticola* in its root means it has important feed and medicinal values. *H. ammodendron* plays an important role in ecological restoration and in the maintenance of ecosystem structure and function. It is predominately located in the desert areas of northwest China (the desert Gobi area of Inner Mongolia, Gansu, Xinjiang, Qinghai, and other provinces), and also in Central Asia and Siberia, Russia. These areas have a typical continental climate with a dry climate, little precipitation, and high evaporation. In addition, the combination of Na and Si can promote the growth of *H. ammodendron* and improve its drought resistance [37]. In conclusion, *H. ammodendron* has formed its unique characteristics and adaptation mechanism in the process of evolution, and has strong resistance to drought, high temperature, and salinity, which is an important resource

for obtaining salt-tolerant genes in plants and has important research value. However, so far, research on *H. ammodendron* has focused on the structural and physiological changes of its responses to salt stress, while the genomic information of *H. ammodendron* and its physiological and molecular mechanisms for adaptation to salinity, remain unclear. *HaASR1* (NCBI accession number: MN602038.1) and *HaASR2* (NCBI accession number: OL908904) were previously cloned from *H. ammodendron* and their functions were analyzed. The overexpression of *HaASR1* decreased the sensitivity of transgenic *Arabidopsis thaliana* to exogenous ABA, reduced the inhibition of stem growth and enhanced drought tolerance [38]. The overexpression of *HaASR2* increased water use efficiency and photosynthetic capacity of transgenic *Arabidopsis thaliana*, and improved drought and salt tolerance [32].

In this work, the salt tolerance of *Arabidopsis* lines overexpressing *HaASR1* was investigated. The results indicated that *HaASR1* from *H. ammodendron* has potential application values for increasing the salt tolerance of grass and crop plants by genetic engineering.

2. Materials and Methods

2.1. Plant Growth Conditions and Salt Treatment

Three transgenic *Arabidopsis* lines, *HaASR1*-OE1, *HaASR1*-OE2, and *HaASR1*-OE3, were already obtained in our previous work [38]. For plate experiments, the half-strength MS medium was used to cultivate seeds of wild-type *Arabidopsis* (WT) and the three transgenic *Arabidopsis* lines. A total of 100 mM NaCl was supplemented to the medium and placed upright at 28 ± 2 °C/23 ± 2 °C (day/night) with a relative humidity of 60–70%, daily photoperiod of 16 h/d, and photon flux density of 1200 μmol $m^{-2}{\cdot}s^{-1}$.

For pot experiments, sterilized turf soil was used to grow plants of WT and transgenic lines of *Arabidopsis* and was watered with nutrient solution every three days. The nutrient solution containing 200 mM NaCl was used to treat WT and three transgenic lines for 15 days. The physiological indexes of the samples were measured 7 days after treatments. Whole seedlings were used for measurements of physiological indexes. The nutrient solution is 1/2 Hoagland nutrient solution and was provided by our laboratory, and the composition mainly includes KNO_3 202.24 g/L, KH_2PO_4 68.00 g/L, $MgSO_4{\cdot}7H_2O$ 123.225 g/L, and $Ca(NO_3)_2{\cdot}4H_2O$ 118.08 g/L.

2.2. Measurement of Physiological Indexes

The shoots and roots of WT, and the three transgenic lines, were used to measure fresh weight (FW) and dry weight (DW). FW was measured immediately after sample collection. After plant samples were dried at 80 °C for 3 days, DW was measured. The difference between fresh and dry weights was used to calculate shoot water content.

The cryoscopic osmometer (OSMOMAT-030, GONOTEC GmbH, Germany) was used to measure the osmotic potential of leaf sap. The readings (mmol·kg^{-1}) were used to calculate osmotic potential (Ψs) in MPa (mega pascal) with the formula: Ψs = −the readings × R × T, where R = 0.008314 and T = 298.8 K.

The contents of proline and betaine in plant leaves were determined using a kit (Suzhou Kemin Biotechnology Co., LTD., Suzhou, China). Disinfected scissors were used to cut plant leaves at the same part and store them at −80 °C for the measurements of proline and betaine contents.

A conductivity meter (EC215, Hanna, Woonsocket, RI, USA) was used to measure the relative membrane permeability (RMP) of young leaf cells. RMP was calculated with the following equation: RMP (%) = S1/S2 × 100%, where S1 and S2 refer to conductivity of leaves and boiled leaves, respectively.

The acetone and alcohol method was used to measure leaf chlorophyll content. Digimizer software was used to measure leaf area. The automatic photosynthetic measuring apparatus (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA, with Li-Cor sample chamber) was used to measure net photosynthetic rate (Pn) and stomatal conductance (Gs) under a photon flux density of 1200 μmol $m^{-2}{\cdot}s^{-1}$, temperature of 28 ± 2 °C, daily photoperiod of

16 h/d, and relative humidity of 60–70%. Net photosynthetic rate divided by transpiration rate (Tr) was used as water use efficiency (WUE).

2.3. Statistical Analysis

SPSS 19.0 was used to conduct one-way analysis of variance (ANOVA) and Duncan's multiple tests were conducted to determine significant differences among means ($p < 0.05$). All experimental results were expressed as means with standard errors (SE) ($n = 6$ or 8).

3. Results

3.1. Overexpression of HaASR1 Improved Salt Tolerance of Arabidopsis Plants

There was no significant difference in growth and development among transgenic lines and WT under normal conditions in half-strength MS medium and soil. After 10 days of 100 mM NaCl treatment, the leaf and primary root growth of transgenic lines were better than that of WT (Figure 1a). After 7 days of 200 mM NaCl treatment, the leaves of WT gradually turned yellow, while most of the transgenic lines were green. After 15 days of 200 mM NaCl treatment, the leaf growth of transgenic lines was better than that of WT, especially OE2 and OE3 which were still green (Figure 1b).

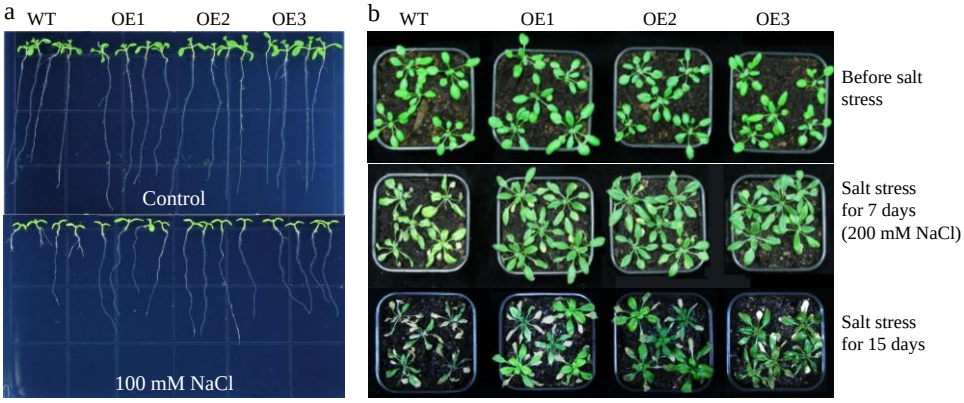


Figure 1. Growth performance of transgenic *Arabidopsis* lines overexpressing *HaASR1*. (**a**) The seedlings were treated with 100 mM NaCl for 7 days, (**b**) plants were treated with 200 mM NaCl.

3.2. Overexpression of HaASR1 Increased Biomass Accumulation in Arabidopsis under Salt Treatment

Compared with the WT, the overexpression of *HaASR1* had no effect on shoot DW, root FW, and root DW of *Arabidopsis* plants under normal conditions; the shoot FW of OE2 and OE3 were significantly higher than that of WT. Compared with the WT, the overexpression of *HaASR1* significantly increased shoot FW, shoot DW, root FW, and root DW of transgenic lines under 200 mM NaCl treatment. The shoot FW of OE1, OE2, and OE3 were 69%, 80%, and 78% higher than that of WT, respectively (Figure 2a); the shoot DW of OE1, OE2, and OE3 were 26%, 26%, and 28% higher than that of WT, respectively (Figure 2b); the root FW of OE1, OE2, and OE3 were 42%, 58%, and 55% higher than that of WT, respectively (Figure 2c); the root DW of OE1, OE2, and OE3 were 29%, 54%, and 51% higher than that of WT, respectively (Figure 2d).

3.3. Overexpression of HaASR1 Maintained Water Content in Arabidopsis under Salt Treatment

There was no significant difference in proline content, betaine content, osmotic potential, and water content among transgenic lines and WT plants under normal conditions. Compared with the WT, the overexpression of *HaASR1* significantly increased proline content, betaine content, and water content of transgenic lines under 200 mM NaCl treatment. The proline contents of OE1, OE2, and OE3 were 18%, 19%, and 12% higher than that of WT, respectively (Figure 3a). The betaine contents of OE1 and OE2 were 21% and 16% higher than that of WT, respectively (Figure 3b). The water contents of OE1, OE2, and OE3 were 48%, 57%, and 54% higher than that of WT, respectively (Figure 3d). The osmotic

potential of transgenic lines was obviously reduced under 200 mM NaCl treatment. The osmotic potential of OE1, OE2, and OE3 were 28%, 26%, and 32% lower than that of WT, respectively (Figure 3c).

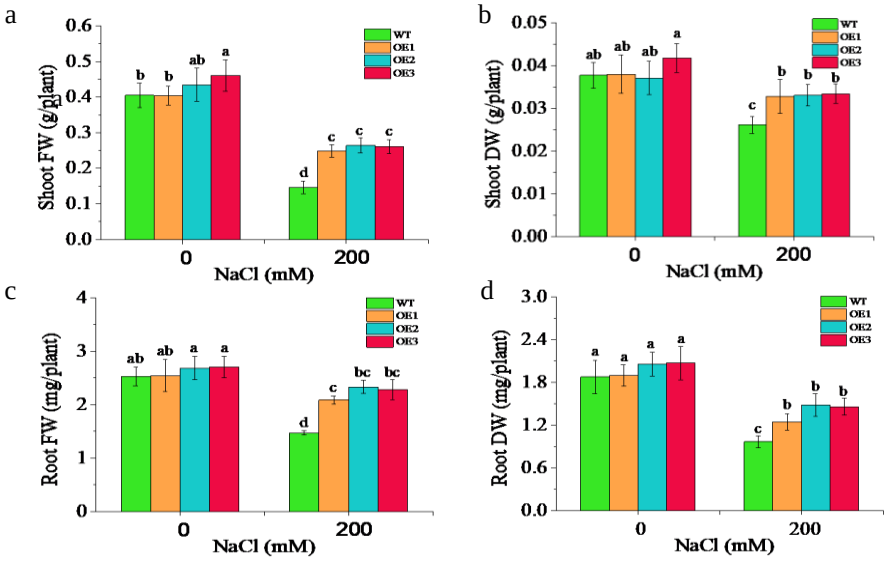


Figure 2. Shoot and root fresh weight and dry weight of transgenic *Arabidopsis* lines overexpressing *HaASR1* treated with 200 mM NaCl. (**a**) Shoot FW, (**b**) shoot DW, (**c**) root FW, (**d**) root DW. Bars represent standard errors (SE) (n = 8), and different lowercase letters represent the significant difference at $p < 0.05$.

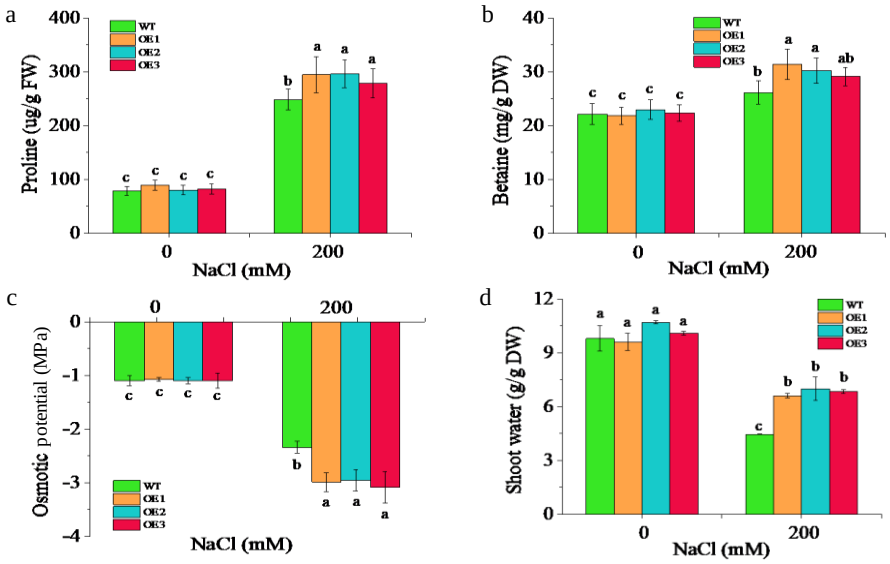


Figure 3. Osmotic potential and water content of transgenic *Arabidopsis* lines overexpressing *HaASR1* treated with 200 mM NaCl. (**a**) Proline, (**b**) betaine, (**c**) osmotic potential, (**d**) shoot water. Bars represent the standard errors (SE) (n = 8), and different lowercase letters represent the significant difference at $p < 0.05$.

3.4. Overexpression of HaASR1 Maintained Membrane Integrity and Improved Chlorophyll Content of Arabidopsis under Salt Treatment

There was no significant difference in the RMP, the contents of chlorophyll *a* and chlorophyll *b*, and leaf area among transgenic lines and WT under normal conditions. Compared with WT, the overexpression of *HaASR1* significantly increased the chlorophyll *a* and chlorophyll *b* content and leaf area of transgenic lines under 200 mM NaCl treatment. The chlorophyll *a* content of OE1, OE2, and OE3 were 38%, 38%, and 53% higher than that

of WT, respectively (Figure 4b). The chlorophyll *b* content of OE1, OE2, and OE3 were 38%, 25%, and 52% higher than that of WT, respectively (Figure 4c). The leaf area of OE1, OE2, and OE3 were 14%, 18%, and 20% higher than that of WT, respectively (Figure 4d). The RMP of transgenic lines was obviously reduced under 200 mM NaCl treatment. The RMPs of OE1, OE2, and OE3 were 24%, 38%, and 29% lower than that of WT, respectively (Figure 4a).

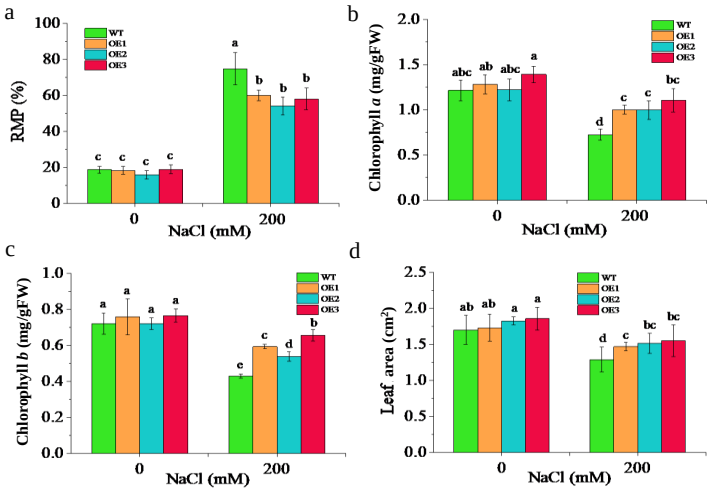


Figure 4. RMP. (**a**) Chlorophyll *a*, (**b**) chlorophyll *b* (**c**), and leaf area (**d**) of transgenic *Arabidopsis* lines overexpressing *HaASR1* treated with 200 mM NaCl. Bars represent standard errors (SE) (n = 6), and different lowercase letters represent the significant difference at $p < 0.05$.

3.5. Overexpression of HaASR1 Increased Photosynthetic Rate of Arabidopsis under Salt Treatment

Compared with the WT, the overexpression of *HaASR1* had no effect on the WUE of transgenic lines under normal conditions. The Pn, Tr and Gs of OE1 and OE2 were higher than that of WT. Under 200 mM NaCl treatment, there was no significant difference in Tr among transgenic lines and WT (Figure 5a). Compared with the WT, the overexpression of *HaASR1* significantly increased the Gs, WUE, and Pn of transgenic lines. The Gs of OE1, OE2, and OE3 were 72%, 50%, and 78% higher than that of WT, respectively (Figure 5b). The WUE of OE1, OE2, and OE3 were 34%, 36%, and 40% higher than that of WT, respectively (Figure 5c). The Pn of OE1, OE2, and OE3 were 57%, 37%, and 65% higher than that of WT, respectively (Figure 5d).

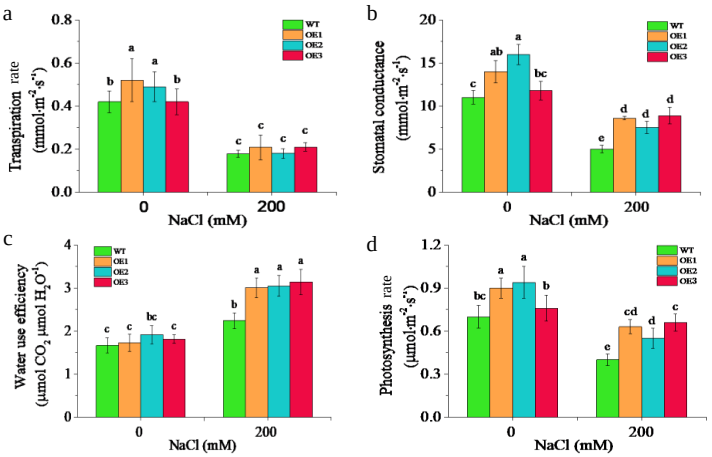


Figure 5. Transpiration rate (**a**), stomatal conductance (**b**), water use efficiency (**c**) and photosynthesis rate (**d**) of transgenic *Arabidopsis* lines overexpressing *HaASR1* treated with 200 mM NaCl. Bars represent standard errors (SE) (n = 6), and different lowercase letters represent the significant difference at $p < 0.05$.

4. Discussion

Plants can gradually adapt to various complex external environments through molecular, morphological and physiological levels after continuous evolution and development. However, different species respond to adverse environments in different ways. Plants' adaptations to environmental changes, including the perception of external stimuli, signal transmission, and stress genes, were expressed so as to make morphological or physiological responses [39]. ASR has been cloned in many Monocotyledons and Dicotyledonae plants, and it can slow down cell damage and maintain osmotic balance under high salt stress [17]. Tissue-specific analysis showed that *HaASR1* and *HaASR2* were mainly expressed in the roots. The sequence homology of *HaASR1* and *HaASR2* is 80%, showing high similarity. An amount of 350 mM NaCl can increase the expressions of *HaASR1* and *HaASR2*, which play an important role in plant response to salt stress [32,38]. However, the gene family of ASR from desert plant species has not been reported.

An increase in salinity causes osmotic stress, rapid water loss of leaves, inhibition of cell elongation and division, and slow plant growth [40]. Plants respond rapidly to osmotic stress in high salinity habitats, which restricts cell expansion and plant growth [41]. The *NAC* gene family plays a leading role in plant resistance to biotic and abiotic stresses. After *OsNAC2* was knocked out in rice, the length of taproot and the quantity of lateral roots were increased obviously [42]. The overexpression of the *GhXND1*-encoded *NAC* transcription factor in *Arabidopsis* reduced the number of xylem conduit cells and the thickness of inter-bundle fibers in transgenic lines [43]. *NAC1* is also involved in plants' lateral root development, and the overexpression of *ZmNAC1* in maize is improved by the lateral root development of inbred lines. At the same time, soybean *GmNAC04*, *GmNAC20*, and *GmN*AC109 can activate the auxin signal and promote the formation of lateral roots [44,45]. The expression of *NHX* can improve plant salt tolerance without a significant effect on fruit development. The overexpression of *AtNHX1* in rapeseed can increase the transgenic lines growth under 200 mM NaCl, and high salinity soil did not affect seed yield and oil quality [46]. The overexpression of *AtNHX1* in tomato made the transgenic lines grow, bloom and bear fruit normally under 200 mM NaCl without effect on the quality of tomato fruits [47]. In this study, the cotyledon size and root length of transgenic *Arabidopsis* lines were higher than those of the WT under 100 mM NaCl. The leaf growth of three transgenic lines were significantly better than that of WT under 200 mM NaCl. Therefore, the overexpression of *HaASR1* could reduce the damage induced by salinity and maintain the growth of plants under salt treatment to some extent.

Phase-soluble penetrants are soluble organic compounds involved in plant cell metabolism, among which the main substances are proline, betaine, polyol, and so on. Under salt stress, small organic molecules accumulate in plants [48]. In rice, osmotic regulation is realized through the synthesis and accumulation of proline, NH_4^+, betaine, and urea. The accumulation of proline increases with the increase in salt content in plant cells, and proline plays three important roles under salt stress: first, as an osmotic regulator to enhance plant resistance to salt stress, then as an organic nitrogen source reserve during the adaptation period to salt stress and, finally, as an important signaling factor in regulating plant cell proliferation and stress resistance gene expression during the adaptation and recovery period [49,50]. Plant cells synthesize proline through the glutamic acid metabolism pathway under osmotic stress [51,52]. In addition, the external application of proline can alleviate salt stress damage [53]. The rapid accumulation of proline is an efficacious defense mechanism for plants against stress [54,55]. Salt stress promotes proline production and its accumulation is proportional to the external osmotic pressure in order to maintain cellular structure and regulate osmotic homeostasis, helping to restore plant water [56,57]. Proline can also enhance plant salt resistance by enhancing antioxidant enzyme activity and plant growth [58]. In addition, the application of exogenous proline can also reduce the damage induced by salinity through enhancing the activity of antioxidant enzymes, reducing the absorption and transport of Na^+ and Cl^- from root to shoot, and enhancing the absorption of K^+ by plants [59]. Betaine is a widespread alkaloid in plants, which can adjust

cell osmotic pressure and keep the integrity of cell membrane and protein structure and function, and it has been widely used in plants to resist stress [60]. Exogenous betaine can improve osmotic substances, the regulation of cell membrane permeability, ion absorption balance and the photosynthetic function of tomato, wolfberry, and barley seedlings under salt stress [61,62]. Proline and betaine are important osmo-regulators of plant cells [63,64]. Cytoplasm substance contents (such as betaine and proline) are increased to maintain the osmotic balance of plant cells in salt stress [65]. The foliar spraying of 25 mM betaine can limit Na^+ accumulation, induce K^+ absorption, maintain a low Na^+/K^+ ratio, and enhance salt tolerance [66]. In this work, the contents of proline and betaine accumulated in transgenic lines were significantly higher than those in WT under salinity, and the osmotic potential in transgenic lines was significantly lower than that of WT, indicating that the proline and betaine accumulated in transgenic lines contributed to improved salt tolerance. In addition, shoot water content of transgenic lines was significantly higher than that of WT under salt treatment, indicating that the accumulation of solutes in transgenic lines contributed to water retention by osmotic regulation.

Chlorophyll is the most vital pigment in photosynthesis. It can transfer absorbed light energy to the reaction center, convert CO_2 and H_2O into organic matter, and release O_2 [67]. To some extent, chlorophyll content directly reflects the strength of a plant's photosynthetic capacity and serves as an important index to measure plant stress resistance [68]. Under salt stress, the reactive oxygen species were accumulated, plasma membranes were peroxidated, thylakoids were destroyed, and chlorophyll was degraded in plants [69]. In this study, the contents of chlorophyll *a* and chlorophyll *b* in transgenic lines under salt treatment were significantly higher than that of WT, especially OE3. The leaf area of transgenic lines was higher than that of WT under salinity, especially OE3. In other words, the reduction in chlorophyll and photosynthetic leaf area of transgenic lines was lower than that of WT under salt treatment, which means they have higher photosynthetic efficiency.

The enhancing of Na^+ and the reduction of cellular water potential in leaves leads to the reduction of stomatal conductance under salt stress [70]. A decreased stomatal conductance causes a reduction in transpiration and water loss, limiting CO_2 supply. Moreover, the stomatal conductance of halophytes largely determines the net photosynthetic rate [71,72]. Meanwhile, a large amount of accumulated ions leads to the destruction of the thylakoid membrane structure and the reduction of mesophyll cell activity under salt stress [73]. Photosynthesis is the basis for the survival of plants in the process of growth and development. Abiotic stress will interfere with a plant's photosynthesis, reduce the absorption and transformation of light energy, and affect carbon assimilation ability, thus inhibiting the growth of plants. Changes in salt concentration and the maintenance of stress will also affect the degree of damage to the photosynthetic system. The chloroplast is the workshop of plant photosynthesis, where a series of energy conversions and the biosynthesis of amino and fatty acids are carried out, and the strength of photosynthesis can be intuitively reflected by the content of chlorophyll [74]. When plants are sustaining salt stress, the activity of the chloroplast lyase is activated to accelerate the decomposition of the chloroplast, so as to mitigate the influences of salt stress on photosynthetic characteristics. A low concentration of salt stress may increase the chlorophyll content, but the content of chlorophyll gradually decreases with a longer stress time and an increase in salt concentration [75]. The chlorophyll content of *Chenopodium quinoa* decreases with an increase in salt concentration [76]. Long-term salt stress can decrease the activities of ribulose diphosphate (RuBP) carboxylase and phosphoenolpyruvate (PEP) carboxylase in chloroplasts, reducing the efficiency of carbon absorption and assimilation, destroying the biosynthesis of pigment proteins in PS system, and accelerating the rate of damage and degradation. Thus, the chloroplast's absorption of light energy and its ability to be converted into chemical energy are weakened, and energy transfer is disrupted. Salt stress affects the reception and transfer of photoelectrons in the photosystem, resulting in insufficient excitation energy in the reaction and severely reducing the conversion of biochemical energy. The absorption and conversion system of plant light energy is damaged, it cannot operate normally, and mem-

brane electron transfer is blocked, thus the potential activity of the photosynthetic system and the efficiency of primary light energy conversion will be affected [76]. In addition, the lamellar structure in the chloroplast tends to decompose under salt stress, which affects the photochemical reaction efficiency and leads to the decline of photosynthetic efficiency. Salt stress significantly affected the chlorophyll fluorescence activity of *Arabidopsis*, and its chloroplast light energy absorption ability, photochemical activity, and electron transfer and light energy conversion abilities were inhibited, and carbon assimilation efficiency was also weakened [77]. In this work, Pn, Gs, and water content of transgenic lines treated with salt stress were significantly higher than that of WT. An increase in net photosynthetic rate and stomatal conductance will help to increase the dry matter accumulation and salt tolerance of plants.

As a desert plant, *H. ammodendron* has undergone morphological changes and evolution in order to adapt to harsh environments. *H. ammodendron* has a strong adaptability to various kinds of adversity, but research on its resistance mechanism is still insufficient. The adaptability of *H. ammodendron* to extreme environments is not only reflected in its morphology, but also in its physiological adaptation as well as its molecular response, signal transduction, and gene regulation [32]. Therefore, research on the stress response mechanism of *H. ammodendron* and the in-depth exploration of stress resistance genes will help us understand the molecular mechanism of plants coping with extreme environments, and provide the research basis for the analysis of desert plants with superb stress resistance. Meanwhile, the research results can also provide numerous genetic resources for the improvement of crop and herbage stress resistance. The results of this work showed that *HaASR1* can improve the salt tolerance of transgenic *Arabidopsis* lines. The investigation of the effects of *HaASR1* overexpression on other abiotic stress tolerances of *Arabidopsis* is helpful in further revealing the biological function of *HaASR1* and its application value. In addition, the function of this gene in Na^+ absorption and transport can be further analyzed by gene knockout or by using tissue-specific promoters to drive *HaASR1* expression. At the same time, the downstream target genes affected by *HaASR1* can also be identified by Chp-Seq and RNA-seq technologies.

5. Conclusions

The overexpression of *HaASR1* significantly increased the growth of *Arabidopsis* plants under salinity and plant tissue water content through enhancing osmotic adjustment ability, maintaining membrane integrity, improving chlorophyll content and leaf area, and thereby enhancing photosynthesis capacity. This study revealed that *HaASR1* from *H. ammodendron* plays a critical role in the plant's adaptation to salt stress and has potential application values for increasing the salt tolerance of grass and crop plants by genetic engineering.

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