Gibberellic acid signaling promotes resistance to saline–alkaline stress by increasing the uptake of ammonium in rice

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Abstract

Gibberellic acid (GA) plays important roles in diverse biological processes in plants. However, its function in the resistance of rice (*Oryza sativa*) to saline-alkaline (SAK) stress is unclear. In this study, we found that SAK stimuli changed the levels of expression of the GA signaling genes. Genetic analyses using the mutants of key GA signaling regulators Slender rice 1 (SLR1) and Dwarf 1(D1) demonstrated that SLR1 negatively regulated the resistance of rice to SAK stress, while D1 positively regulated it, suggesting the GA signaling positively regulates the resistance of rice to SAK. A previous study showed that SLR1 interacts with Phytochrome interacting factor-like 14 (PIL14) to regulate the resistance of rice to salt stress. However, *PIL14* overexpressor (*OX*) plants exhibited a similar response to SAK as the wild-type (WT) controls. Further analyses revealed that SLR1 interacted with and inhibited the activation of transcription of IDD10 and bZIP23. Furthermore, IDD10 interacted with bZIP23 to activate *Ammonium transporter 1;2* (*AMT1;2*), and *slr1*, *IDD10 OX* and *bZIP23 OX* accumulated more ammonium (NH_4^+) than the WT plants. In addition, the *bzip23 T-DNA* mutant was more sensitive to SAK, while *bZIP23 OX* was less sensitive compared with the WT. This suggested that bZIP23 is similar to IDD10, which positively regulates the resistance of rice to SAK. Taken together, this data proved that GA signaling promotes the resistance of rice to SAK by modulating the uptake of NH_4^+ in rice mediated by SLR1-IDD10-bZIP23.

Introduction

Salt stress seriously affects plant growth and development, which affects the entire growth cycle of plants(Yu et al., 2020), including seed germination, the development of plant growth, and plant physiological and biochemical characteristics(Kohler et al., 2009; Kuiper et al., 1990; Lombardi et al., 1998; Tang & Newton, 2005). Salt and alkali stress can reduce the soil osmotic potential, cause ion imbalances, disrupt physiological processes, inhibit plant growth, and reduce crop quality and yields(Capula-Rodríguez et al., 2016). Therefore, the exploitation and utilization of saline-alkali land has become one of the important ways to enhance agricultural benefits.

Plant hormones are small molecules that regulate plant growth and development, as well as responses to changing environmental conditions. Plants can regulate and coordinate growth and stress tolerance by modifying the production of hormones and their distribution or signal transduction(E. H. Colebrook et al., 2014). Gibberellic acid (GA) is one of the phytohormones that is necessary for plant growth and development. In recent years, with the advancement of molecular genetics and functional genomics, significant progress has been made in the identification of upstream GA signaling components and *trans*- and *cis*-acting factors that regulate downstream GA-responsive genes in higher plants(Sun & Gubler, 2004). The GA signaling pathway has also been well studied. GA binds to its soluble, nuclear receptor known as GIBBERELLIN INSENSITIVE DWARF 1 (GID1), which causes a conformational change in the protein that promotes its association with the N-terminal domain of the DELLA protein, which, in turn, enables its interaction with an SCF ubiquitin ligase. This results in the ubiquitination of DELLA, which targets it for degradation via the 26S proteasome(Ueguchi-Tanaka et al., 2005). In addition, Dwarf 1 is

involved in the regulation of a GA signaling pathway in rice (*Oryza sativa*) that depends on a GTP-binding protein(Ashikari, 1999). DELLA proteins are named because they form a subgroup of the GRAS family of proteins that is a conserved domain at the N terminus that is highly conserved in *Arabidopsis thaliana* and other species, including rice (Slender Rice 1, SLR1), wheat (*Triticum aestivum*) (Reduced height, Rht), barley (*Hordeum vulgare*) (Slender 1, SLN1) and maize (*Zea mays*) (Dwarf 8, D8)(Hirano et al., 2012; C. Jiang & X. Fu, 2007). Although DELLA proteins are key negative regulators in the GA signaling pathway(Zhong et al., 2021), there is no evidence that they bind directly to gene promoters. Some evidence indicates that they will interact with transcription factors (TFs) and form complexes. The complex sometimes acts as a transcriptional activator (Hirano et al., 2012) or as an inhibitor through sequestration(de Lucas et al., 2008; S. Feng et al., 2008).

DELLAs have been shown to interact with and inhibit the activity of key regulatory proteins to modulate plant development(Hong et al., 2012; Josse et al., 2011). For example, the physical interaction between INDETERMINATE DOMAIN 1 (IDD1) and DELLA and the accumulation of DELLA triggered by IDD1 promotes seed maturation during the later stage of development(Feurtado et al., 2011). DELLAs interact with PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) and PIF4 to inhibit their ability to interact with target gene promoters, thereby blocking their ability to inhibit transcription(Suhua Feng et al., 2008). Additionally, the bioactive levels of GA are reduced upon salt treatment in *A. thaliana* seedlings(Achard et al., 2006). The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of DELLA protein to affect the metabolism of GA(Achard, Gong, et al., 2008). In *A. thaliana*, reactive oxygen species are involved in the regulation of root growth mediated by DELLA and the promotion of stress growth(Achard, Renou, et al., 2008). In rice, PHYTOCHROME-INTERACTING FACTOR-LIKE14 (OsPIL14) interacts with SLR1 to integrate light and GA signals to precisely control seedling growth under salt stress(Mo et al., 2020). The ubiquitin binding protein DOMINANT SUPPRESSOR of KAR2 (OsDSK2a) regulates the growth and development of rice under salt stress by regulating the level of ELONGATED UPPERMOST INTERNODE (EUI) protein, a regulatory factor of gibberellin metabolism(Wu et al., 2020). The reduction in levels of GA and therefore, its signaling, has been shown to contribute to the restriction of plant growth following exposure to several stresses.

The tolerance of plants to salt is a complex regulatory network in which different molecules are involved in complex crosstalk. Soil salinization disrupts the ion homeostasis in plants(Ruiz et al., 2016). The supply of ammonium (NH_4^+) improved the salt tolerance of the plant by restricting the accumulation of sodium (Na^+) and improving potassium (K^+)/Na^+ homeostasis in shoots(Miranda et al., 2017). NH_4^+ can induce tolerance to salt in sorghum (*Sorghum bicolor*) plants by synergistically activating Na^+ homeostasis in sorghum plants under salt stress(Miranda et al., 2017). In *A. thaliana*, overexpression of the NH_4^+ transporter gene from the extreme halophyte *Puccinellia tenuiflor*a (*PutAMT1;1*) significantly improved salt tolerance during the early root growth stage after seed germination(Bu et al., 2019). More recently, we demonstrated that *phytochrome B* (*phyB*) mutants exhibited improved tolerance to salt-alkaline (SAK) stress by activating the uptake of NH_4^+(Jung et al., 2023). In addition, SLR1 can also interact with GROWTH-REGULATING FACTOR 4 (OsGRF4) and inhibit the interaction between OsGRF4

and OsGIF (GRF-interacting factor), thus, inhibiting the absorption and assimilation of nitrogen (N) in plants(Li et al., 2018). GA inhibits the growth and development of plant branches by promoting the degradation of N-mediated tiller growth response 5 (NGR5) protein to promote the expression of target genes(Wu et al., 2020). However, it is not clear how the GA signal regulates the transport of NH_4^+ under SAK stress in rice.

In this study, we analyzed the function of GA signaling on the resistance of rice to SAK. Our results revealed that SLR1 negatively regulated the resistance of rice to SAK. Conversely, D1 promoted the resistance of rice to SAK stress. In addition, our results indicated that Slender Rice 1 (SLR1) interacted with IDD10 and bZIP23 to inhibit their activation of transcription, and IDD10 interacted with bZIP23 to activate the level of expression of *Ammonium transporter 1;2* (*AMT1;2*) to improve the uptake of NH_4^+ in rice. *AMT1;2* promoted the resistance of rice to SAK stresses. In addition, IDD10 and bZIP23 promoted the resistance of rice to SAK stress. These results reveal the molecular mechanism that underlies the regulation of NH_4^+ uptake by GA signaling and provides insight to improve resistance to SAK stress in rice.

Materials And Methods

Plant growth

The (WT) rice cultivars (*Oryza sativa* L. Japonica cultivars Zhonghua 11, Nipponbare, Hwayoung and Dongjin) and the *slr1*, *d1*, *idd10*, *IDD10 OX*, *bzip23*, *bZIP23 OX*, *PIL14 OX* lines were used in this study. All the rice plants were grown in liquid 0.5X MS media for 2–3 days. The plants were grown in liquid 0.5X MS media with 80 mM of $NaHCO_3$ for 10 days to determine their tolerance to SAK. After 10 days, the growth of rice roots was calculated. All the rice were grown in environmental chambers at 22–24℃, 60% relative humidity, and a 12/12 h light/dark photoperiod. *Nicotiana benthamiana* plants were cultured in environmental chambers at 22–24℃, 60% relative humidity (RH), and a 16/8 h light/dark photoperiod for 4 weeks before use.

Mutants And Transgenic Plants

The preparation of *idd10*, *IDD10 OX*, *bzip23*, *bZIP23 OX*, *PIL14 OX* was previously described(Mo et al., 2020; Xiang et al., 2008; Xuan et al., 2013). The coding regions of *slr1* and *d1* were sequenced to generate *slr1* and *d1*. The *slr1* mutants were obtained by CRISPR/Cas9 (Baige Gene Technology, Jiangsu, China). The *d1* mutant is a natural mutant that could be identified by a map cloning strategy(Fujisawa et al., 1999)(Fig. 1c).

Yeast Two-hybrid Assay

The full-length coding sequences of *IDD10* and *SLR1* were amplified by PCR, verified by sequencing, and cloned into pGAD424, while the *IDD10* and *bZIP23* open reading frame (ORF) was cloned into the pGBT9 vector. The pair of plasmids were further transformed in the yeast strain Y2HGold. Yeast cells that harbored the pair of plasmids were grown on SD/-Trp /-Leu and SD/-Trp /-Leu /-His plates. The primers used for cloning *bZIP23*, *SLR1*, and *IDD10* are listed in Table S2.

Bimolecular Fluorescence Complementation (Bifc) Assay

The coding sequences of *IDD10*, *bZIP23*, *SLR1* and *SLR1-C* were cloned into the fluorescent protein vectors pXNGW and pXCGW. IDD10-cCFP and bZIP23-nYFP, bZIP23-nYFP and SLR1-cCFP or IDD10-cCFP and SLR1-C-nYFP and fusion vectors were constructed and co-transformed into tobacco leaves using the *Agrobacterium tumefaciens* strain GV3101 for the BiFC assay(Kim et al., 2009). H2B-RFP was used as a nuclear marker, and pXNGW and pXCGW vectors with no target genes were used as the negative control. The yellow fluorescence protein (YFP) signals were observed under a confocal microscope (Olympus FV1000, Tokyo, Japan) 36 to 48 h after infiltration.

Co-immunoprecipitation (Co-ip) And Western Blotting

Co-immunoprecipitation (Co-IP) and western blotting
bZIP23 and *IDD10* were cloned into the pGD3GGm vectors to generate the constructs. The IDD10-Myc and bZIP23-GFP or IDD10-GFP and SLR1-Myc were co-expressed in *N. benthamiana* leaves. Each interaction pair was mixed with P19 at a 5:5:3 ratio to infiltrate the tobacco leaves. The protein was extracted after 36 h, and the Co-IP assay was performed as previously described(Kim et al., 2009). Approximately 2 g of protein from each leaf sample was collected, and the total protein was extracted using extraction buffer (150 mM NaCl, 2.5 mM Tris–HCl, 1 mM EDTA, 10% glycerol, 0.1% NP-40, 2% polyvinylpolypyrrolidone (PVPP), 10 mM dithiothreitol (DTT), and 1X protease inhibitor cocktail). Western blotting was performed using the following primary antibodies: anti-GFP antibody (ABclonal Technology, Wuhan, China) and anti-Myc antibody (ABclonal Technology). The signal was detected using an ECL Western Blotting Detection System (GE Healthcare, Piscataway, NJ, USA).

Determination of NH_4^+ and total N contents

The NH_4^+ content in the roots and shoots of 7-day-old rice seedlings was measured using an F-kit (Roche, Basel, Switzerland) according to the manufacturer's instructions(Oliveira et al., 2002).

ChIP-qPCR assay

bZIP23 OX seeds were used for the ChIP-quantitative PCR (qPCR) assays. The calli from homozygous *35S::GFP* and *35S:: bZIP23-GFP* mutant plants were used for ChIP-qPCR assays as previously described. The immunoprecipitated DNAs were analyzed by ChIP-PCR to identify the bZIP23 binding region. The

immunoprecipitated DNA was normalized by each input DNA in ChIP-PCR(Je et al., 2010). The primers used for ChIP-qPCR are listed in Table S2.

Transactivation assay

Effector (*p35S::bZIP23*, *p35S::IDD10*, and *p35S:SLR1*), reporter (*pAMT1;2*), and internal control (*p35S::LUC*) plasmids were used to co-transform protoplasts (Yamaguchi *et al*., 2010). A GUS activity analysis was performed as previously described (Xuan et al., 2013). A luciferase assay was performed using a Luciferase Assay Kit (Promega, Madison, WI, USA), and PEG-mediated transformation and luciferase activity assays were performed as previously described(Yoo et al., 2007). The primers used for the transient expression assay are listed in Table S2.

Statistical analyses

Statistical analyses were conducted using Prism 8.0 software (GraphPad, San Diego, CA, USA) with a one-way analysis of variance (ANOVA) to compare significant differences between multiple groups. In addition, a Student's *t*-test was used to compare the differences between the two groups. All the data were expressed as the mean ± SE (n = 3). Differences between the groups were considered significant with at least $P < 0.05$.

Results

GA signaling positively regulates the resistance of rice to SAK

To investigate the molecular mechanism of the resistance of rice to SAK, we analyzed the transcriptome data of rice genes for their response to SAK stimuli(Liu et al., 2021) and found that there were abundant genes related to GA signaling (Fig. 1a). This suggests that GA signaling is involved in the resistance of rice to SAK (Table S1). Both SLR1 and D1 are key regulators in GA signaling. SLR1 is a repressor of GA signaling(Davière & Achard, 2013), while D1 is a positive regulator(Ashikari & Yoshimura, 1999; Ashikari, 1999). Subsequently, the SAK stress responses were examined in *slr1* and *d1* mutants(Fujisawa et al., 1999) by evaluating primary root growth. A *SLR1* CRISPR/Cas9-induced genome editing mutant was used. The sequencing results showed that there was a 1bp deletion in *slr1* at the exon (Fig. 1b). *slr1*, *d1* and their corresponding wild-type (WT) plants were grown in liquid 0.5X MS media with 80 mM of sodium bicarbonate ($NaHCO_3$) for 10 days. The seedling growth assay that utilized 80 mM of $NaHCO_3$ revealed that the *slr1* plants were more tolerant to SAK stress (Fig. 1d–g), while the *d1* plants were more sensitive than the WT to SAK stress (Fig. 1h–k). This suggests that GA signaling positively regulates the resistance of rice resistance to SAK.

PIL14 overexpressor (OX) exhibited a similar response to SAK compared with the wild-type plants

A previous study reported that SLR1 interacts with Phytochrome interacting factor-like 14 (PIL14) to regulate the resistance of rice to salt stress, which extended the knowledge of how crops adapt to saline

environments(Mo et al., 2020). Therefore, the *PIL14* overexpressor (*OX*) plants were treated with 80 mM of $NaHCO_3$ to examine their responses to SAK stress. The results indicated that the root growth of *PIL14 OX* was similar to that of the WT plants in response to SAK stress (Fig. 2a–d). This suggests that PIL14 is not involved in regulating the resistance of rice to SAK stress.

Slr1 Interacts With Idd10 To Inhibit The Activation Of Transcription

To further investigate the mechanism of SLR1 on the resistance of rice to SAK, the interacting proteins of SLR1 were screened by a yeast two-hybrid (Y2H) assay. The Y2H assay showed that AD-SLR1 interacts with BD-IDD10 (Fig. 3a). Next, the interaction between SLR1 and IDD10 was confirmed using multiple methods. Bimolecular fluorescence complementation (BiFC) showed that SLR1 interacts with IDD10 in the nucleus of tobacco (*Nicotiana benthamiana*) plants (Fig. 3b). Subsequently, a co-immunoprecipitation (Co-IP) assay was performed to confirm the interaction between IDD10-GFP and SLR1-Myc in tobacco leaves. The total protein was immunoprecipitated with an anti-GFP antibody and immunoblotted with anti-GFP and anti-Myc antisera. The results indicated that IDD10 interacts with SLR1 (Fig. 3c). DELLA proteins can bind to IDD family TFs(Blanco-Touriñán & Alabadí, 2020). Additionally, our previous research showed that the *idd10* mutants were sensitive to SAK stresses (Fig. 3d-g). In contrast, *IDD10 OX* exhibited tolerance to SAK stresses (Fig. 3h-k). This result indicated that IDD10 positively regulated the resistance of rice to SAK.

A previous study showed that IDD10 directly activates *AMT1;2* by promoter binding(Xuan et al., 2013). SLR1 is known to interact with TFs to modulate the activation of their transcription(Davière & Achard, 2013). A transient assay was performed to analyze the effects of SLR1 on IDD10 by co-expressing *35S:SLR1*, *35S:IDD10* and *pAMT1;2-GUS* constructs in the protoplasts. The results indicated that IDD10 activated *pAMT1;2-GUS*, while SLR1 did not. However, the co-expression of SLR1 and IDD10 inhibited the activation of *pAMT1;2-GUS* mediated by IDD10 (Fig. 3l). In addition, the contents of NH_4^+ were calculated in the ZH11, *slr1*, HW and *idd10* roots. The results showed that *idd10* accumulated less NH_4^+ than its WT control HW, while *slr1* accumulated more NH_4^+ than its WT control ZH11 (Fig. 3m, n).

Slr1 Interacts With Bzip23 To Inhibit The Activation Of Transcription

The Y2H assay results revealed that SLR1 also interacted with bZIP23 in rice (Fig. 4a). Previous studies have shown that the TF OsbZIP23 is an important regulator of abiotic stress response and the abscisic acid (ABA) signal transduction pathway and is involved in the resistance of rice to stress(Zong et al., 2016). A BiFC assay revealed that SLR1 interacted with bZIP23 in the nucleus where the YFP signal colocalized with a nuclear marker H2B-RFP (Moreland & Hereford, 1987) (Fig. 4b). A Co-IP assay that utilized SLR1-Myc and bZIP23-GFP indicated that SLR1 and bZIP23 interacted in planta (Fig. 4c).

To further investigate the function of bZIP23 in the resistance to SAK stress, the *bzip23 T-DNA* mutant, *bZIP23 OX* and WT were treated with 80 mM of $NaHCO_3$. After all the plants were grown for 10 days, the

bzip23 mutant plants were more sensitive, while the root growth of *bZIP23 OX* was more tolerant to SAK stress than that of the WT plants (Fig. 4d–g).

IDD10 interacts with bZIP23 to activate AMT1;2

SLR1 interacted with IDD10 and bZIP23. In addition, the interaction between IDD10 and bZIP23 was investigated. A Y2H assay revealed that IDD10 interacts with bZIP23 (Fig. 5a). A BiFC assay indicated that IDD10 interacts with bZIP23 in the nucleus (Fig. 5b). A Co-IP assay was then performed by expressing IDD10-Myc and bZIP23-GFP in tobacco leaves, which indicated that IDD10 interacts with bZIP23 in planta (Fig. 5c).

Previously, we reported that the TF IDD10 activates the expression of *AMT1;2* expression to improve the uptake of NH_4^+(Xuan et al., 2013). Next, the possibility of activation of *AMT1;2* by bZIP23 was tested. A transient assay that co-expressed *35S: bZIP23*, *35S:IDD10* and *pAMT1;2-GUS* constructs in the protoplasts showed that both bZIP23 and IDD10 activate *pAMT1;2-GUS*, and the co-expression of bZIP23 and IDD10 has additive effects on the activation of *pAMT1;2-GUS* compared with the expression of either bZIP23 or IDD10 alone (Fig. 5d). To examine the direct binding of bZIP23 to the *AMT1;2* promoter, a chromatin immunoprecipitation (ChIP) assay was performed. The promoter sequences were analyzed before the ChIP assay. The results indicated that the putative bZIP23 binding motifs were located within 38 kb of the *AMT1;2* promoter (Fig. 5e). The ChIP-PCR results indicated that bZIP23 directly bound the P1 region in *AMT1;2* promoter (Fig. 5f). In addition, the contents of NH_4^+ were calculated in the WT, *bzip23 mutant* and *bZIP23 OX* plants. The results indicated that the *bzip23* mutant accumulated less NH_4^+, while *bZIP23 OX* accumulated more compared with the WT plants (Fig. 5g).

SLR1 interacts with IDD10 to inhibit the activation of IDD10 transcription. Therefore, the effects of SLR1 on bZIP23 were examined. The transient assay results showed that bZIP23 activated *pAMT1;2-GUS*, but the co-expression of SLR1 and bZIP23 inhibited the activation of *pAMT1;2-GUS* that was mediated by bZIP23. In addition, the expression of SLR1 also inhibited the activation of *pAMT1;2-GUS* that was mediated by IDD10-bZIP23 (Fig. 5h).

Amt1 Positively Regulates The Resistance Of Rice To Sak

IDD10 and bZIP23 positively regulate the tolerance of rice to SAK, as well as the expression of *AMT1;2*. Therefore, the patterns of growth of *AMT1 RNAi* and *AMT1;1* overexpression plants under SAK stress were examined. *AMT1 RNAi* in which *AMT1;1*, *AMT1;2* and *AMT1;3* were knocked down(Li et al., 2016) was more sensitive to SAK compared with the WT plant DJ. In parallel, the *AMT1;1 OX* plants were more tolerant to SAK compared with the WT plants (Fig. 6a–d).

Discussion

GA plays important roles in plant growth and development. The GA signaling pathway has been shown to be involved in plant growth and development under various stresses, such as low temperature and salt. However, its function in the resistance of rice to SAK stresses remains unclear.

Ga Signaling Positively Regulates The Resistance Of Rice To Sak

The functional mechanism of GA signaling in the resistance of rice to SAK is unclear. In this study, we analyzed the role of two key genes in GA signaling in the resistance of rice to SAK. After 10 days of treatment of the *slr1* and *d1* mutants in 80 mM of $NaHCO_3$, we found that the *slr1* mutant was more resistant to SAK than the WT (Fig. 1d–g). Conversely, the *d1* mutant was significantly more susceptible to disease than the WT (Fig. 1h–k). SLR1 is a key negative regulator in GA signal transduction(Cao et al., 2005; Cheng et al., 2004), suggesting that GA signaling plays a positive role in the resistance of rice to SAK. Additionally, a previous study reported that SLR1 can interact with PIL14 to improve salt tolerance in rice(Mo et al., 2020). Therefore, we tested whether PIL14 has the same effect on SAK tolerance. Similarly, the *PIL14 OX*s were treated with 80 mM of $NaHCO_3$ for 10 days to calculate the root growth of the plants. The results showed that the growth of *PIL14 OX*s plants after treatment was basically the same as that of the control plants (Fig. 2), suggesting that PIL14 may not play a role in the resistance of rice to SAK. This suggests that SLR1 regulates the tolerance of rice to SAK using other molecular modules.

The Slr1-idd10-bzip23 Transcription Complex Regulates The Resistance Of Rice To Sak

SLR1 interacts with IDD10 (Fig. 3a–c). IDD10 is closely related to the root growth of rice, which activates the expression of *AMT1; 2* to promote the uptake of NH_4^+ in rice roots(Xuan et al., 2013). Additionally, IDD10 positively regulates the resistance of rice to SAK(Jung et al., 2023). However, SLR1 negatively regulates the resistance of rice to SAK (Fig. 1d–g). Since SLR1 is a transcriptional suppressor that interacts with TFs to degrade them or inhibit their DNA-binding activity (Jiang & Fu, 2007), the effect of SLR1 on IDD10 was analyzed. The transient assay indicated that IDD10 activated *AMT1;2*, while SLR1 did not. In addition, *idd10* accumulated less NH_4^+ than its WT, while slr1 accumulated more than its WT (Fig. 3m, n). This suggested that the regulation of SAK in rice by SLR1 is a complex regulatory network. Interestingly, a multiple interaction analysis showed that SLR1 interacts with bZIP23 (Fig. 4a–c), and the *bzip23* mutant was more sensitive to SAK tolerance than the WT (Fig. 4d–g). bZIP23 is a key TF in the ABA signaling pathway, which plays an important role in the response of rice to stress(Xiang et al., 2008; Zong et al., 2016). The growth and development of plants in SAK soil primarily depend on seed germination, which is closely related to ABA and GA signaling(Colebrook et al., 2014). In *A. thaliana*, INDUCER OF CBF EXPRESSION1 (ICE1) antagonizes ABSCISIC ACID INSENSITIVE5 (ABI5) and DELLA activity to maintain appropriate levels of ABA signaling to participate in the regulation of seed germination(Hu et al., 2019). This suggests that the SLR1-bZIP23 regulatory complex could be similar in rice and *A. thaliana*.

There is extensive crosstalk between salt stress signaling pathways and other stress signaling pathways(Van Zelm et al., 2020). Since SLR1 interacts with both IDD10 and bZIP23, the interaction between IDD10 and bZIP23 was analyzed. The data indicate that IDD10 interacts with bZIP23 in the nucleus (Fig. 5a–c). Additionally, the transient assay confirmed that bZIP23 and IDD10 activate *AMT1;2*, and the co-expression of bZIP23 and IDD10 has additive effects on the activation of *AMT1;2* compared with the expression of either bZIP23 or IDD10 alone (Fig. 5d). The *bzip23* mutant accumulated less NH_4^+, while *bZIP23 OX* accumulated more compared with the WT plants (Fig. 5g). Subsequently, the effects of SLR1 on bZIP23 were examined. The data showed that SLR1 also inhibited the activation of *AMT1;2* mediated by bZIP23 (Fig. 5h). In conclusion, GA regulates the resistance of rice to SAK through the SLR1-IDD10-bZIP23 complex.

Ga Signaling Promotes Resistance To Sak Stress By Increasing The Uptake Of Ammonium In Rice

The data shows that SLR1 interacts with IDD10 and bZIP23 to inhibit their transcriptional activity, and the downstream *AMT1;2* will not be activated (Figs. 3–5). Therefore, this was also confirmed by the data that *IDD10 OX*, *bZIP23 OX*, *AMT1 OX*, *slr1* plants had a strong tolerance to SAK (Figs. 1, 3, 4, 6). However, in the presence of GA, the GA-bound GID1 receptor interacts with the DELLA protein. This interaction triggers the degradation of DELLA protein and the action of GA by the SCFGID2/SLY1 proteasome pathway(Hirano et al., 2010). This indicates that the SLR1-IDD10-bZIP23 transcription complex will not be formed, and AMT1 activity will be activated when the amount of GA in the environment is increased. In addition, IDD10 interacts with bZIP23 to activate the expression of *AMT1;2* to promote the uptake of NH_4^+ (Fig. 5d). This suggests that GA signaling promotes the resistance to SAK stress by increasing the uptake of ammonium in rice. According to incomplete statistics of the Food and Agriculture Organization (FAO) and UNESCO, the total area of SAK land in the world is 954.38 million ha, and the salinization of land severely inhibits the growth and development of plants. Thus, the salinization and secondary salinization of soil in the world restricts the sustainable development of agriculture. Previously, we reported that PhyB enhances resistance to SAK stress in rice by mediating the uptake of NH_4^+(Jung et al., 2023). In this study, we identified a molecular mechanism by which GA signaling is involved in the uptake of NH_4^+, which suggests that the DELLA proteins SLR1 and AMT1 genes could be used to improve the resistance of rice to SAK stress. This study describes a method to improve the stress resistance of rice by increasing the efficiency of NH_4^+ absorption and provides a new concept for breeding an efficient new generation of Green Revolution varieties.

Declarations

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Author Contributions

These authors contributed equally to this work.

Ethics approval and consent to participate

Doesn’t apply.

Consent for publication

Doesn’t apply.

Competing interests

The authors declare that they have no confict of interest.

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Figures

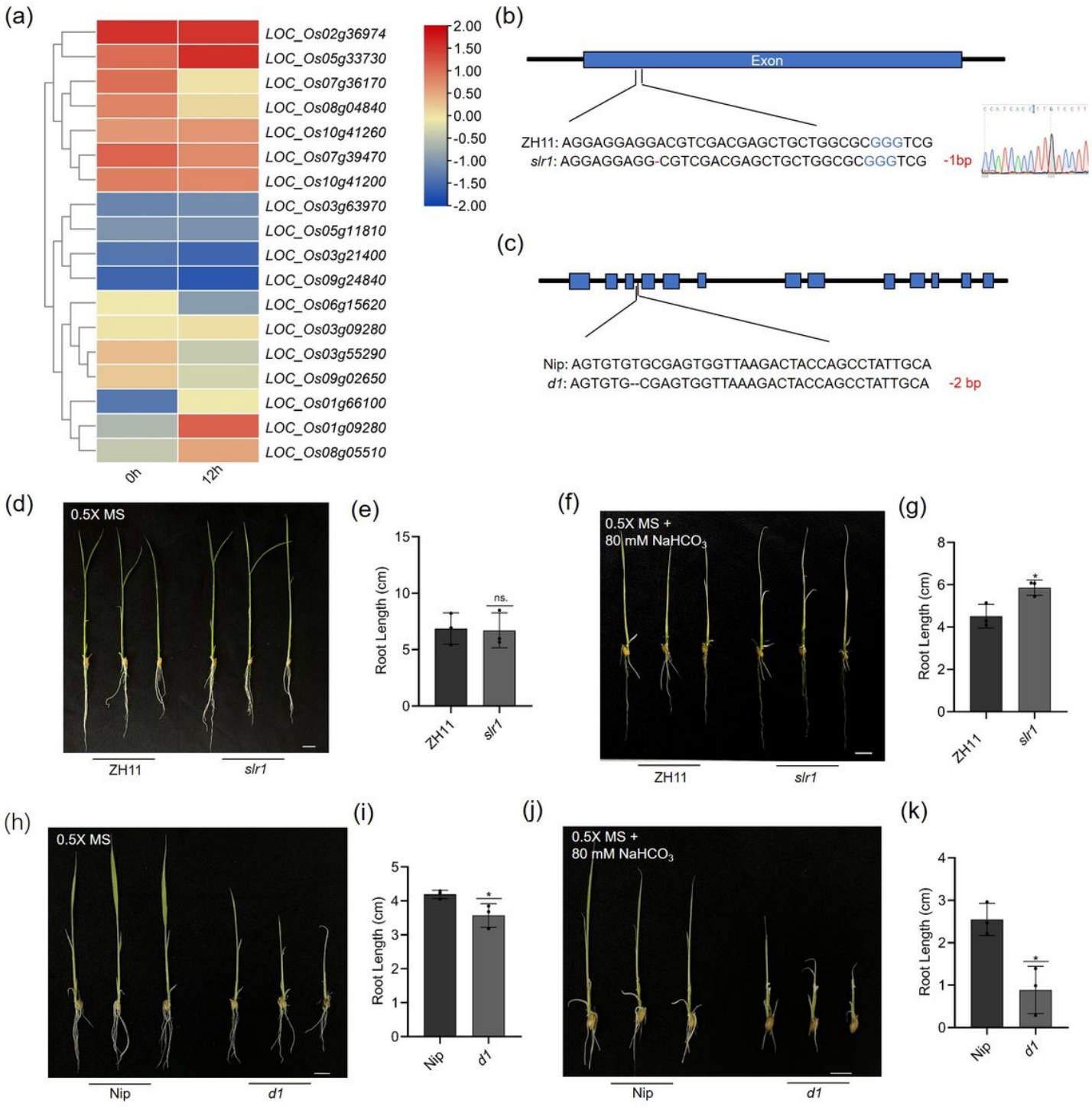

Figure 1

GA signaling positively regulates the resistance of rice to SAK. (a) A heat map of GA signal-related genes in the rice SAK transcriptome. (b) 1 bp was deleted at the exon in the CRISPR/Cas9-induced *slr1* mutant. (c) 2 bp was deleted at the positions of 1,003 and 1,004 in the *d1* mutant. The blue boxes represent exons. (d) (f) (h) (j) *slr1*, d1 and their corresponding wild-type plants were grown on 0.5X MS, 0.5X MS + 80 mM $NaHCO_3$ (saline–alkaline stress) for 10 days. (e) (g) (i) (k) The inhibition of primary root growth was calculated from the plants grown under saline–alkaline stress conditions. The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked

with different letters. GA, gibberellic acid; MS, Murashige & Skoog; $NaHCO_3$, sodium bicarbonate; SAK, saline-alkaline.

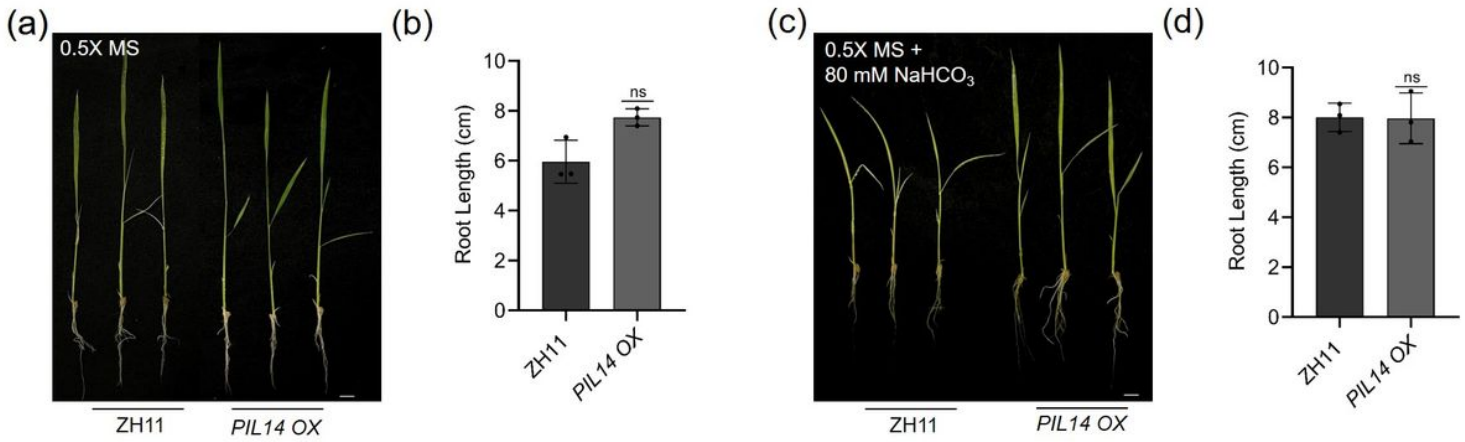

Figure 2

The responses of *PIL14* OX to saline–alkaline stress. (a) (c) *PIL14* OX and wild-type plants were grown on 0.5X MS, 0.5X MS + 80 mM $NaHCO_3$ (saline–alkaline stress) for 10 days. (b) (d) The inhibition of primary root growth was calculated from the plants grown under saline–alkaline stress conditions. The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked with different letters. MS, Murashige & Skoog; $NaHCO_3$, sodium bicarbonate.

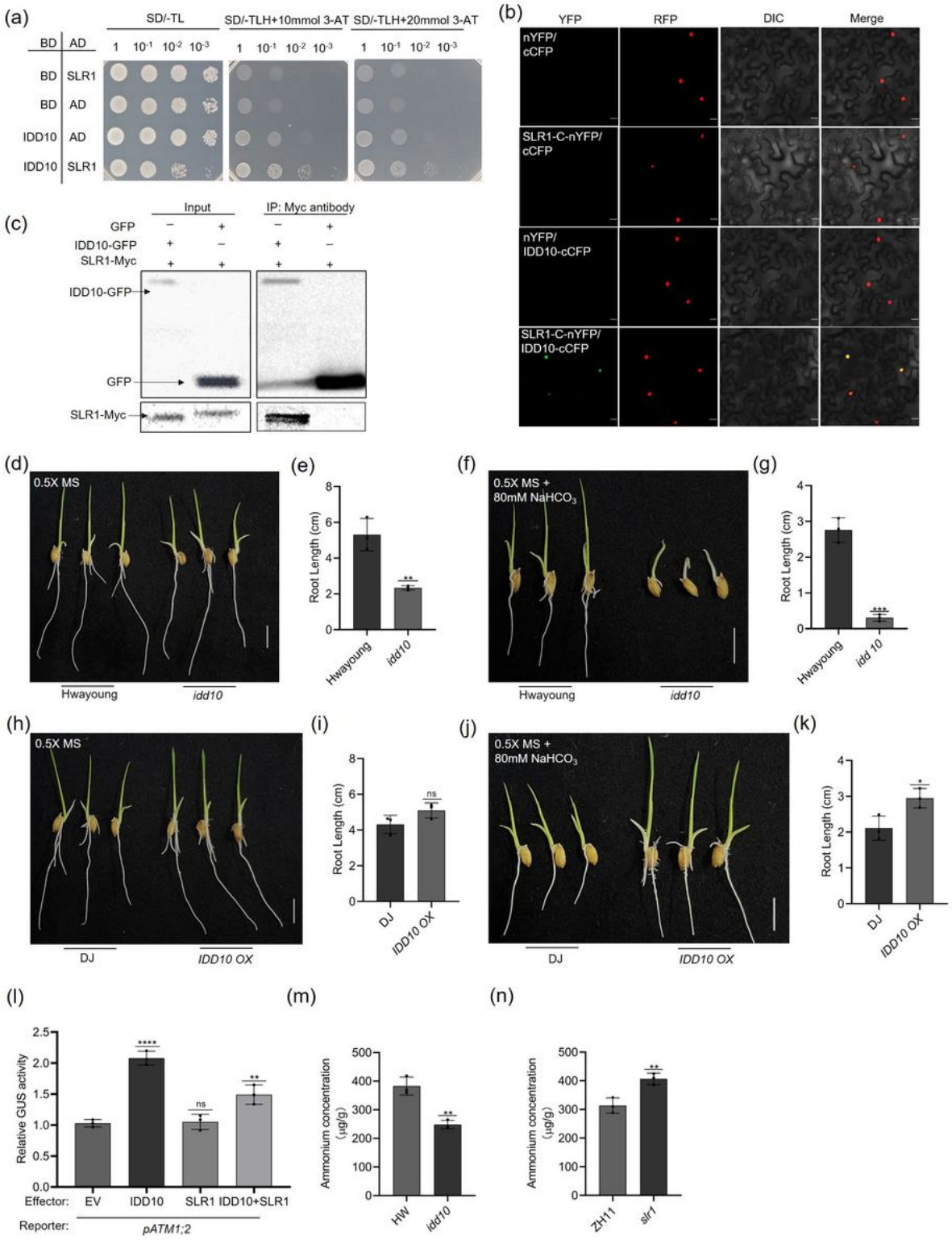

Figure 3

SLR1 interacted with IDD10 and the responses of *IDD10* plants to saline–alkaline stress. (a) A Y2H assay was performed to assess the interaction between BD-IDD10 and AD-SLR1. Yeast cells transformed with the vectors indicated were grown on SD/-Trp -Leu or SD/-Trp -Leu -His + 10 mM and 20 mM 3-AT. EV: empty vector. (b) A BiFC assay was performed to analyze the interaction between IDD10 and SLR1-C. The reconstruction of YFP from IDD10-cCFP + nYFP, cCFP + SLR1-C-nYFP, or IDD10-cCFP + SLR1-C-nYFP was

imaged. H2B-RFP was used as the nuclear marker, and differential interference contrast (DIC) and merged images were captured. Bar = 20 μm. (c) A Co-IP assay was performed to assess the interaction between IDD10-GFP and SLR1-Myc. Anti-GFP antiserum was used for immunoprecipitation, and anti-GFP or anti-Myc antibodies were used for the WB analysis. (d) (f) (h) (j) *idd10*, *IDD10* OX and their corresponding wild-type plants were grown on 0.5x MS, 0.5x MS + 80 mM $NaHCO_3$ (SAK stress) for 10 days. (e) (g) (i) (k) Primary root growth inhibition was calculated from the plants grown under SAK stress conditions. The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked with different letters. (l) A transient expression assay was performed to analyze the effects of IDD10, SLR1 and IDD10 + SLR1 on the activation of the *AMT1;2* promoter (*pAMT1;2*). The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked with different letters. (m) (n) Contents of NH_4^+ in the roots of HW, *idd10*, ZH11and *slr1* plants were measured. BiFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; GFP, green fluorescent protein; MS, Murashige & Skoog; $NaHCO_3$, sodium bicarbonate; NH_4^+, ammonium; SAK, saline-alkaline; WB, western blot; Y2H, yeast two-hybrid; YFP, yellow fluorescent protein.

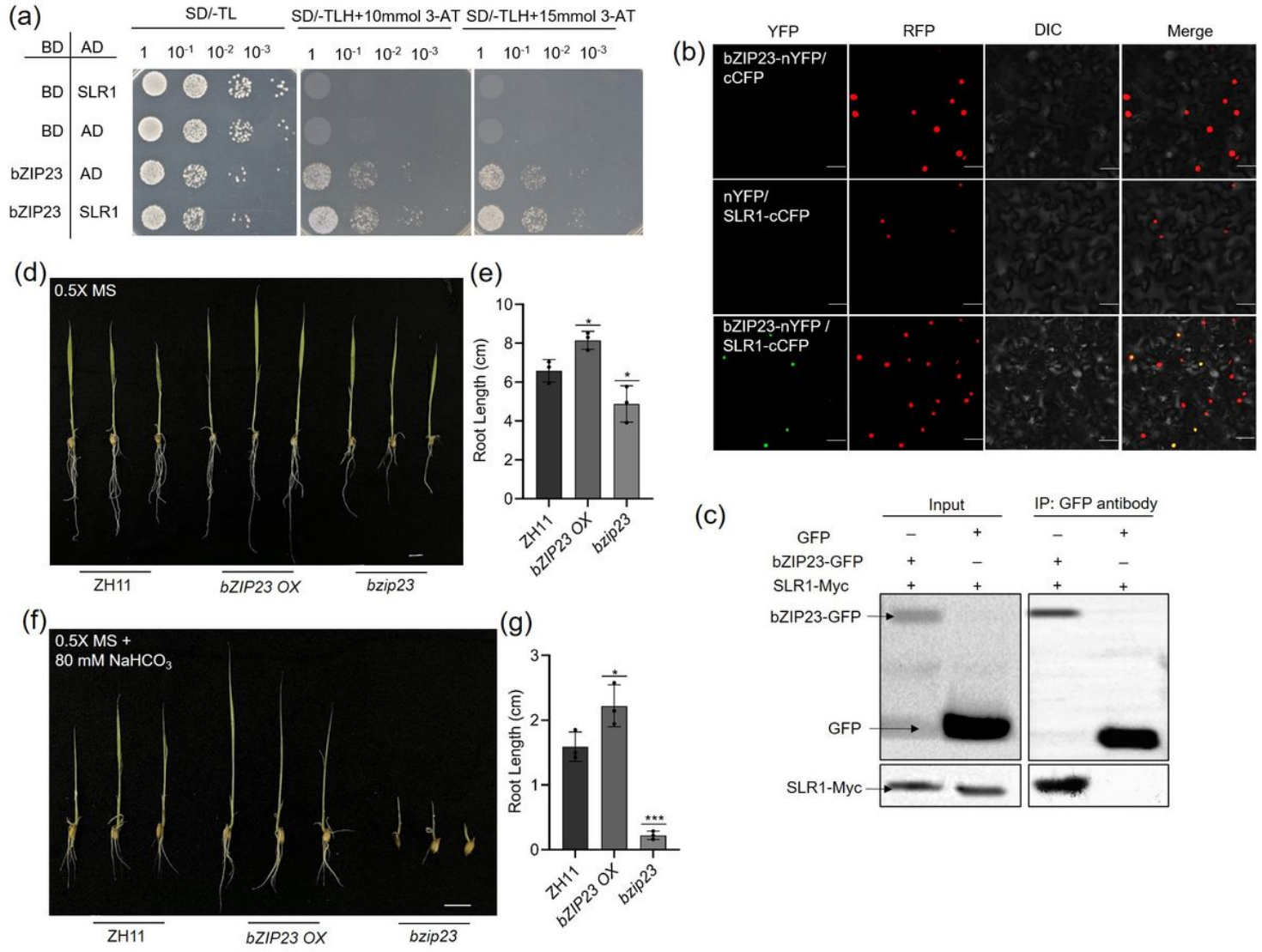

Figure 4

SLR1 interacted with bZIP23 and the responses of *bZIP23* plants to saline–alkaline stress. (a) A Y2H assay was performed to assess the interaction between BD- bZIP23 and AD-SLR1. Yeast cells transformed with the vectors indicated were grown on SD/-Trp -Leu or SD/-Trp -Leu -His + 10 mM and 15 mM 3-AT. EV: empty vector. (b) A BiFC assay was performed to analyze the interaction between bZIP23 and SLR1. The reconstruction of YFP from SLR1-cCFP + nYFP, cCFP + bZIP23-nYFP, or SLR1-cCFP + bZIP23-nYFP was imaged. H2B-RFP was used as the nuclear marker, and differential interference contrast (DIC) and merged images were captured. Bar = 20 μm. (c) A Co-IP assay was performed to assess the interaction between bZIP23-GFP and SLR1-Myc. Anti-GFP antiserum was used for immunoprecipitation, and anti-GFP or anti-Myc antibodies were used for a WB analysis. (d) (f) *bzip23*, *bZIP23* OX and their corresponding wild-type plants were grown on 0.5X MS, 0.5X MS + 80 mM of $NaHCO_3$ (saline–alkaline stress) for 10 days. (e) (g) The inhibition of primary root growth was calculated from the plants grown under saline–alkaline stress conditions. The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked with different letters. 3-AT, 3-aminotriazole; BiFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; GFP, green fluorescent protein; $NaHCO_3$, sodium bicarbonate; WB, western blot; Y2H, yeast two-hybrid.

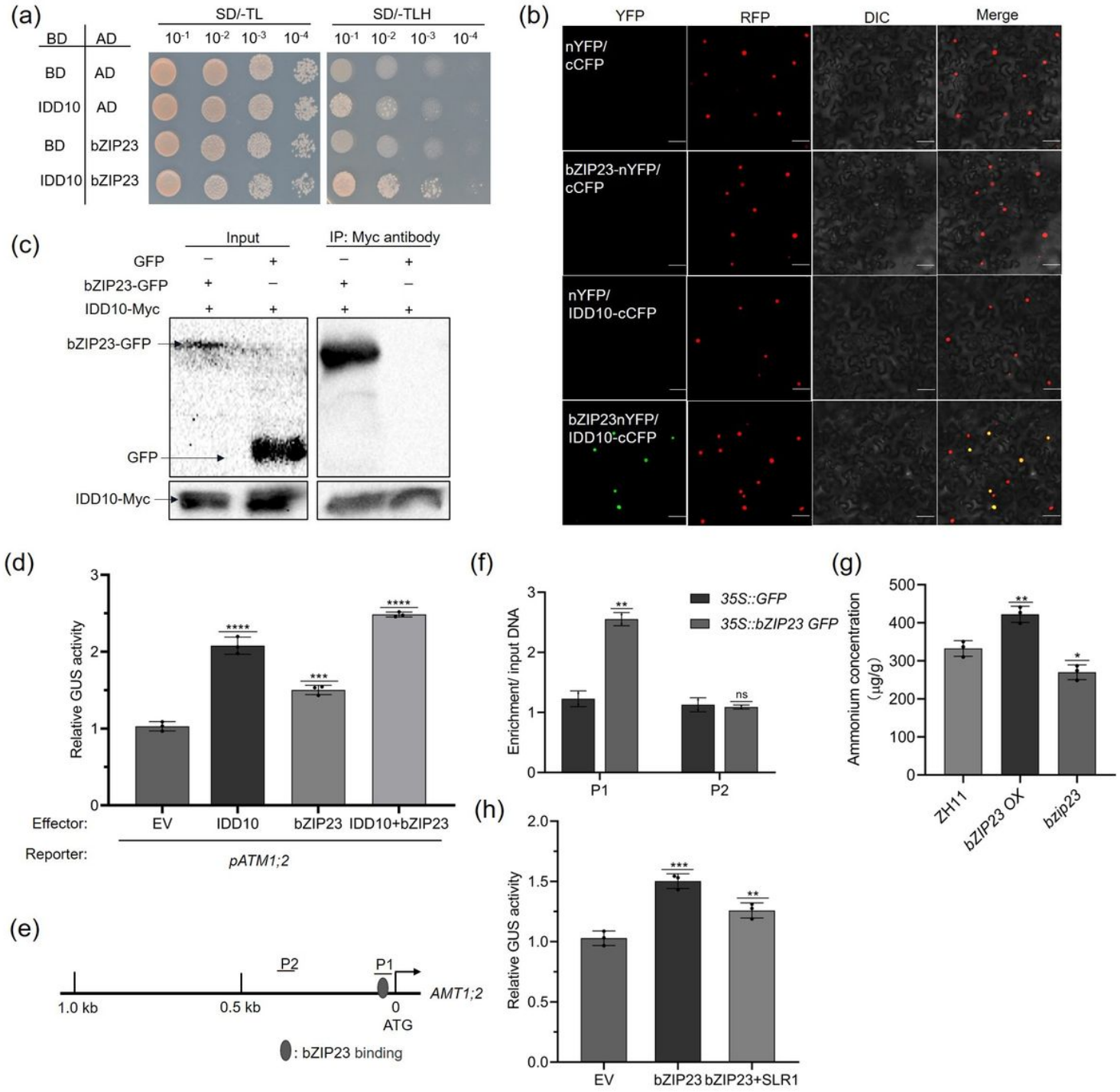

Figure 5

IDD10 interacted with bZIP23. (a) A Y2H assay was performed to assess the interaction between BD-IDD10 and AD-bZIP23. Yeast cells transformed with the vectors indicated were grown on SD/-Trp -Leu or SD/-Trp -Leu -His. EV: empty vector. (b) A BiFC assay was performed to analyze the interaction between bZIP23 and SLR1. The reconstruction of YFP from cCFP + nYFP, IDD10-cCFP + nYFP, cCFP + bZIP23-nYFP, or IDD10-cCFP + bZIP23-nYFP was imaged. H2B-RFP was used as the nuclear marker, and differential interference contrast (DIC) and merged images were captured. Bar = 20 μm. (c) A Co-IP assay was performed to assess the interaction between bZIP23-GFP and IDD10-Myc. Anti-Myc antiserum was

used for immunoprecipitation, and anti-GFP or anti-Myc antibodies were used for a WB analysis. (d) A transient expression assay was performed to analyze the effects of IDD10, bZIP23 and IDD10 + bZIP23 on the activation of the *AMT1;2* promoter (*pAMT1;2*). The experiments were repeated at least three times. Significant differences among more than two groups at $P < 0.05$ are marked with different letters. (e) (f) Schematic diagram showing the position of bZIP23 binding in the *AMT1;2* promoter of the probe used to detect ChIP. The gray oval represents bZIP23 binding, and the letters P(1, 2) indicate the positions. (g) NH_4^+ contents in the roots of wild-type, *bZIP23 OX*, and *bzip23* plants were measured. (h) A transient expression assay was performed to analyze the effects of bZIP23 and bZIP23+SLR1 on the activation of *AMT1;2* promoter (*pAMT1;2*). The experiments were repeated at least three times. Significant differences among more than two groups at *P < 0.05* are marked with different letters. BiFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; GFP, green fluorescent protein; NH_4^+, ammonium; WB, western blot; Y2H, yeast two-hybrid; YFP, yellow fluorescent protein. ChIP, chromatin immunoprecipitation.

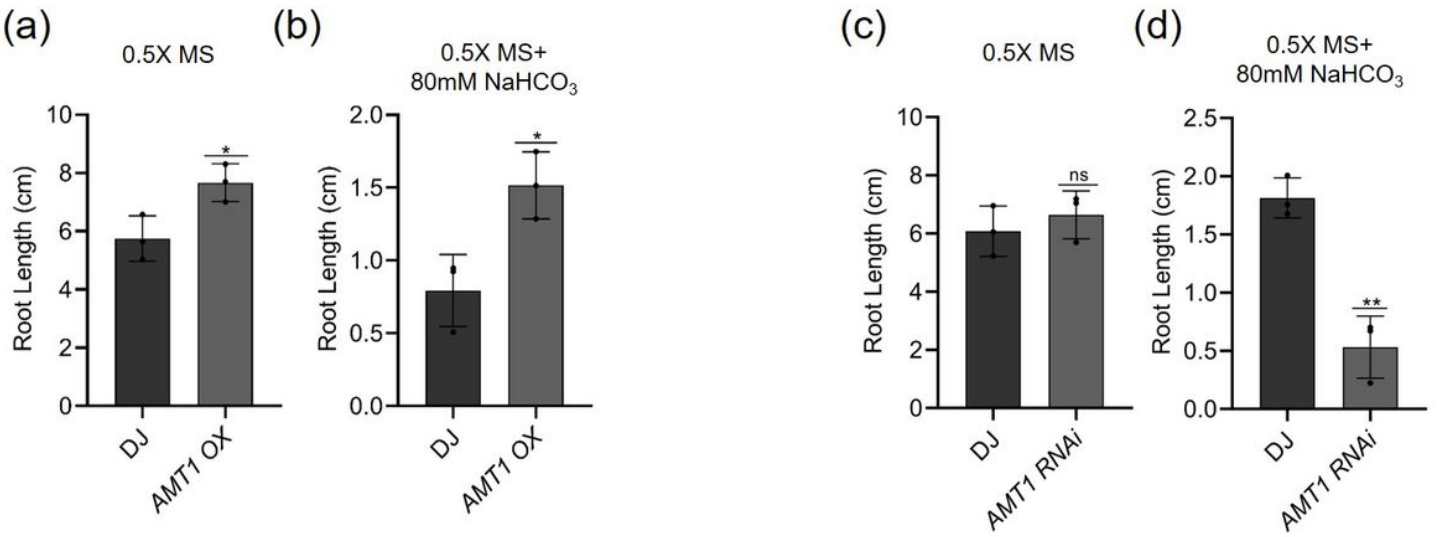

Figure 6

The responses of *AMT1* plants to saline–alkaline stress. (a) (b) (c) (d) The inhibition of primary root growth was calculated from the plants grown under SAK stress conditions. The experiments were repeated at least three times. Significant differences among more than two groups at *P < 0.05* are marked with different letters. SAK, saline-alkaline.

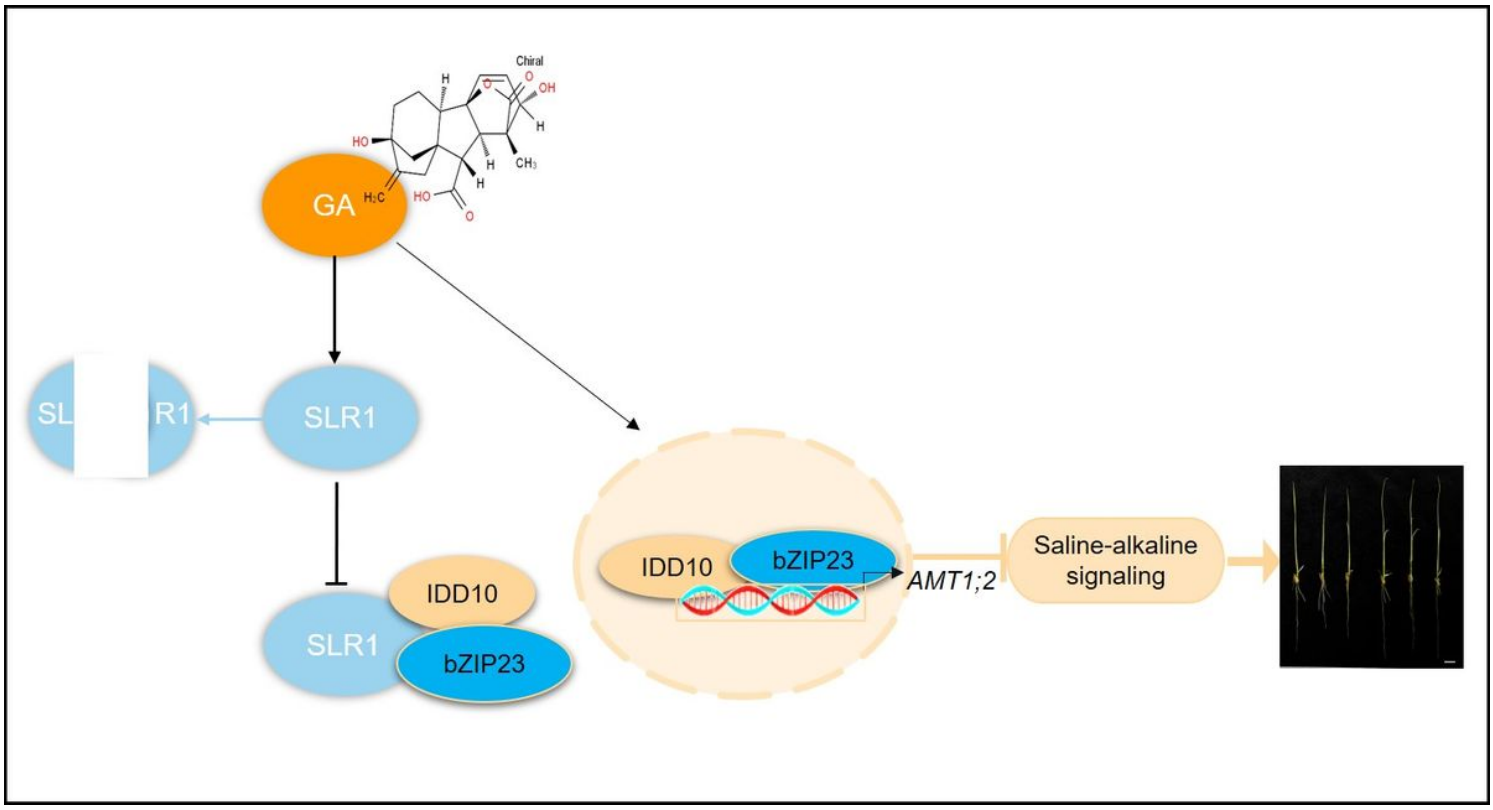

Figure 7

The regulatory effects of SLR1 on the uptake of NH_4^+ and stress responses. SLR1 interacts with bZIP23 and IDD10 to inhibit their transcription activity. SLR1 is degraded when the GA gas is increased. When the GA gas is increased, SLR1 degrades, thereby inhibiting the function of the downstream transcription complex formed by SLR1-IDD10-bZIP23. In the nucleus, bZIP23 interacts with IDD10 to activate the expression of *AMT1;2* to promote the uptake of NH_4^+. The accumulation of NH_4^+ improves the resistance of rice to SAK stress. bZIP23 and IDD10 promote the resistance of rice to SAK stress, respectively. GA, gibberellic acid; NH_4^+, ammonium; SAK, salt-alkaline.

Supplementary Files

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