Arabinose biosynthesis is critical for salt stress tolerance in Arabidopsis

Chunzhao Zhao1,2, Omar Zayed2,3, Fansuo Zeng3, Chaoxian Liu4, Ling Zhang5,6, Peipei Zhu6,7, Yunus E. Tuncil7, W. Andy Tao6, Nicholas C. Carpita8,9 and Jian-Kang Zhu1,2

1CAS Center for Excellence in Molecular Plant Sciences, Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, Shanghai 201602, China; 2Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA; 3State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin 150040, China; 4Maize Research Institute, Southwest University, Chongqing 400715, China; 5Jilin Provincial Key laboratory of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences, Changchun, Jilin 130033, China; 6Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA; 7Food Engineering Department, Ordu University, Ordu 52200, Turkey; 8Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA; 9Purdue Center for Plant Biology, Purdue University, West Lafayette, IN 47907, USA

Authors for correspondence:
Chunzhao Zhao
Tel: +86 21 57078274
Email: czzhao@psc.ac.cn
Jian-Kang Zhu
Tel: +86 21 57078201
Email: jkzhu@sibs.ac.cn

Received: 30 December 2018
Accepted: 16 April 2019

New Phytologist (2019)
doi: 10.1111/nph.15867

Key words: Arabidopsis, arabinogalactan protein, arabinose, cell wall integrity, root elongation, salt stress.

Summary

- The capability to maintain cell wall integrity is critical for plants to adapt to unfavourable conditions. l-Arabinose (Ara) is a constituent of several cell wall polysaccharides and many cell wall-localised glycoproteins, but so far the contribution of Ara metabolism to abiotic stress tolerance is still poorly understood.
- Here, we report that mutations in the MUR4 (also known as HSR8) gene, which is required for the biosynthesis of UDP-Arap in Arabidopsis, led to reduced root elongation under high concentrations of NaCl, KCl, NaNO3, or KNO3.
- The short root phenotype of the mur4/hsr8 mutants under high salinity is rescued by exogenous Ara or gum arabic, a commercial product of arabinogalactan proteins (AGPs) from Acacia senegal. Mutation of the MUR4 gene led to abnormal cell–cell adhesion under salt stress. MUR4 forms either a homodimer or heterodimers with its isoforms. Analysis of the higher order mutants of MUR4 with its three paralogues, MUR1, DUR, MEE25, reveals that the paralogues of MUR4 also contribute to the biosynthesis of UDP-Ara and are critical for root elongation.
- Taken together, our work revealed the importance of the Ara metabolism in salt stress tolerance and also provides new insights into the enzymes involved in the UDP-Ara biosynthesis in plants.

Introduction

l-Arabinose (Ara) is a plant-specific monosaccharide that occurs in 10–20% of noncellulosic cell wall polysaccharides in Arabidopsis (Rautengarten et al., 2017). Ara is found in arabinans and type I arabinogalactans, which are the major components of the pectic wall polymer rhamnogalacturonan-I (RG-I), and constitutes critical residues in the rhamnogalacturonan II (RG-II) variant of homogalacturonan for cross-linking with boron (Caffell & Mohns, 2009). Ara residues are found in hemicellulosic glucuronoxarabinoylan (GAX) and the xyloglucan of Solanaceous species (Carpita & Gibeaut, 1993; Peña et al., 2008; Scheller & Ulvskov, 2010; Schultink et al., 2013, 2014). In addition to polysaccharides, many glycoproteins, such as extensins and leucine-rich repeat extensins, some secreted CLE peptides, and ubiquitous proteoglycan arabinogalactan proteins (AGPs), are also arabinosylated (Showalter, 1993; Velasquez et al., 2011; Okamoto et al., 2013; Borassi et al., 2016; Marzol et al., 2018).

Ara has two ring forms, the furanose (Araf) and the pyranose (Arap) form. In most Ara-containing components in plants, Ara occurs in the furanose form. However, Ara is initially synthesized as a form of UDP-Arap either via a de novo pathway or via a salvage pathway, then UDP-Arap is converted to UDP-Araf in the cytosol (Konishi et al., 2007; Kotake et al., 2016). The de novo synthesis of UDP-Arap is initiated from the conversion of UDP-glucose (UDP-Glc) to UDP-glucuronic acid (UDP-GlcA) catalysed by UDP-Glc dehydrogenases (UGDs; Klinghammer & Tenhaken, 2007); UDP-glucuronic acid decarboxylases (UXSs) subsequently catalyse the conversion of UDP-GlcA to UDP-xylose (UDP-Xyl; Kuang et al., 2016). The conversion of UDP-Xyl to UDP-Arap is catalysed by UDP-Xyl 4-epimerases (UXEs) in the Golgi lumen (Burget et al., 2003). The synthesized UDP-Arap is transported from the Golgi lumen to the cytosol, where a UDP-Ara mutase (UAM) interconverts UDP-Arap to UDP-Araf, which is transported back into the Golgi lumen to be incorporated into Ara-containing polysaccharides (Konishi et al., 2007; Rautengarten et al., 2017). In Arabidopsis, the mutant mur4
(mur4) with a lower level of cell wall Ara was shown to be defective in UXE1 (Burget & Reiter, 1999; Burget et al., 2003). In the mur4 mutant, the Ara content in the cell wall of rosette leaves is reduced by c. 50% (Burget & Reiter, 1999; Li et al., 2007). That 50% of the Ara remains in the mur4 mutant indicated that other enzymes were involved in the biosynthesis of UDP-Ara.

A salvage pathway recycles Ara that is released during the turnover of Ara-containing polymers and glycoproteins in the cell wall (Dolezal & Cobbett, 1991; Sherson et al., 1999; Behmuller et al., 2016). Free arabinoses are phosphorylated by two C-1 arabinokinases, ARA1 and ARA2, and are then converted to UDP-Arap by a UDP-sugar pyrophosphorylase (NDP) with a broad substrate specificity (Kotake et al., 2004, 2007; Behmuller et al., 2016). Mutations in ARA1 or in both the ARA1 and ARA2 genes result in an increase in cytoplasmic Ara content compared with the wild-type (Dolezal & Cobbett, 1991; Behmuller et al., 2016).

A study showed that two members of UDP-galactose (UDP-Gal) epimerases (UGE1 and 3) displayed both UDP-Xyl 4-epimerase and UDP-Glc 4-epimerase activities in vitro (Kotake et al., 2009). Unlike the mur4 mutant, however, the uge1 uge3 double mutant does not contain a reduced Ara content (Rosti et al., 2007), although AGP synthesis is compromised (Seifert et al., 2002).

Previous studies have shown that high sugar response 8 (hsr8), an allele of the mur4 mutant, displays glucose-hypersensitive hypocotyl elongation and development under dark conditions (Li et al., 2007; Seguela-Arnaud et al., 2015). Li et al. (2007) found that the glucose-hypersensitive phenotype of hsr8 mutant can be rescued by boric acid, indicating that the altered cell wall is the cause of the glucose-hypersensitive phenotype of hsr8 mutant plants. Boric acid also restores wild-type stature and stem tensile strength in the low cell wall fucose mutant muras1, implicating RG-II-borate complexes as contributors to cell integrity (O’Neill et al., 2001; Ryden et al., 2003). Genetic studies have indicated that the mutations in PRL1 (Pleiotropic Regulatory Locus 1) or the MED25 and MED8 subunits of the Mediator complex suppress the glucose-hypersensitive phenotype of hsr8 mutant (Li et al., 2007; Seguela-Arnaud et al., 2015).

In this study, we performed a genetic screen for mutants that are hypersensitive to salt stress in Arabidopsis and identified several mutants that showed abnormal root elongation under salt stress. One mutant, in which the UDP-D-xylene 4-epimerase1 (UXE1) gene (MUR4) is mutated, was characterized in this study. Our results showed that mutations in the MUR4 gene, which is required for the biosynthesis of UDP-Ara, led to a reduced root elongation under salt stress, a phenotype that could be recovered by applying exogenous Ara. In addition, we demonstrated that the three paralogues of MUR4 also contributed to the biosynthesis of UDP-Ara and are essential for the maintenance of root elongation in Arabidopsis.

Materials and Methods

Plant materials and growth conditions

All Arabidopsis plants in this study were on the Columbia background. mur4-1 (CS8568), hsr8-2 (SALK_010548), dur (SALK_143736), murl (CS877404), meec25 (SALK_025826), prl1 (SALK_039427), aba2-1, and abi1-1 mutants were obtained from Arabidopsis Biological Resource Center (ABRC, Ohio State University, Columbus, OH). ara1-2 ara2-1 double mutant and hpgt1-1 hpgt2-1 hpgt3-1 triple mutant have been described previously (Ogawa-Ohnishi & Matsubayashi, 2015; Behmuller et al., 2016). med25 hsr8-1, med8 hsr8-1, and mur4-1 prl1-1 were provided by Dr Michael W. Bevan (Li et al., 2007; Seguela-Arnaud et al., 2015). Plants were grown at 23°C with a long-day light cycle (16 h light : 8 h dark).

Mapping by whole-genome resequencing

snrk2.1/3/4/5/6/7/8/9/10 nonuple (2W) mutant seeds (Fujii et al., 2011) were subjected to EMS mutagenesis. For whole-genome resequencing-based mapping, the 20-2 mutant was backcrossed to 2W. In the F2 generation, c. 120 plants with slower root elongation under salt stress were collected and mixed together for genomic DNA isolation using the DNeasy Plant Maxi kit (Qiagen). Whole genomic DNAs were sequenced by Illumina. Resequencing reads from 2W and 20-2 were aligned to the TAIR10 reference genome using BWA (Li & Durbin, 2009). SAM files were generated and converted to BAM files. PCR duplications were removed using MarkDuplicates.jar in Picard (http://broadinstitute.github.io/picard/). HaplotypeCaller in GATK (McKenna et al., 2010) was used to identify SNPs in both wild-type and mutant samples. A five-SNP window was used to calculate the ratio of mutations. Based on the mutation ratio of each window, a line plot was generated along all five chromosomes. The peak position was marked on the chromosome. The SNPs near the peak position were selected to identify the causal mutations.

Construction of plasmids

To examine the expression pattern of MUR4, MEE25, DUR and MURL in tissues, a 2-kb fragment of the promoter sequence of each gene was amplified and cloned into the pMDC162 vector, which contains a β-glucuronidase reporter gene. The constructs were transformed into wild-type plants. The constructs 35S::MUR4::YFP, 35S::MURL::YFP, 35S::DUR::YFP, and 35S::MEE25::YFP were generated by amplifying the coding sequence of each gene and cloning them into the pDONR207 ENTRY vector using BP clonase II (Life Technologies). LR clonase II was used for the recombination of fragments to the destination vector pEarleyGate 101. For split luciferase assay, the coding sequences of MUR4, DUR, MURL, and MEE25 were recombined into pEarleyGate vectors that were modified from the original pCAMBIA-nLUC and pCAMBIA-cLUC vectors (Chen et al., 2008). Primers used for constructs are listed in Supporting Information Table S1.

Microscopic observation of root morphology

To observe root morphology, 5-d-old seedlings were transferred from Murashige and Skoog (MS) medium to 100 mM NaCl medium and grown for an additional 2 d. The root tips were
excised and the elongation zones were observed with bright field optics on a Zeiss Axio Observer D1 microscope. For scanning electron microscopy, roots were frozen in liquid nitrogen and then sputter-coated and examined with an XL 30 FEG (Philips) cryoscanning electron microscope.

Monosaccharide analysis

The rosette leaves from 35-d-old plants or the roots of 15-d-old seedlings were collected for monosaccharide analysis. Cell walls were isolated as described previously (Mertz et al., 2012). In brief, the ground samples were suspended in a buffer with 50 mM Tris (pH 7.2) and 1% sodium dodecyl sulphate (SDS). After being vortexed and centrifuged at 2500 g for 5 min, the supernatants were removed and the pellets were resuspended in the same buffer with and heated at 65°C for 20 min. The pellets were washed three times with 50°C water, three times with 50% ethanol, and three times with water at ambient temperature. Cell walls were freeze dried for 2 d before analysis of monosaccharides. About 2 mg of cell wall of each sample was hydrolysed in glass vials with 2 M trifluoroacetic acid (TFA) supplemented with 0.5 μM of myo-inositol as an internal standard. The samples were incubated at 120°C for 90 min. After the samples cooled to room temperature, 0.5 ml of tert-butyl alcohol was added, and the samples were dried overnight under a stream of air at 40°C. A 0.6-ml volume of a solution containing 0.5 ml of fresh 20 mg ml⁻¹ NaBH₄ in dimethyl sulphoxide (DMSO) plus 0.1 ml of 1 M NH₄OH was added. The samples were incubated at 45°C for 90 min and then neutralised with 100 μl of glacial acetic acid. A 100-μl volume of 1-methylimidazole and 0.75 ml of anhydrous acetic anhydride were added, and the mixtures were incubated at 45°C for 30 min. Finally, 1.5 ml of H₂O and 1 ml of dichloromethane were added, and the samples were centrifuged at 600 g for 3 min and washed five times with H₂O. The CH₂Cl₂ was evaporated at 40–45°C, and the samples were re-dissolved in 0.5 ml of CH₃Cl₂ for GC-MS analysis. Samples were analysed by gas–liquid chromatography on a 0.25-mm × 30-m SP-2330 column (Supelco, Bellefonte, PA, USA).

Quantitative real-time RT-PCR analysis

Total RNA was extracted from whole seedlings using a plant RNA kit (Omega Bio-tek, Norcross, CA, USA) according to the manufacturer’s instructions. 2 μg RNA was treated with DNase using a Turbo DNA-free kit (Applied Biosystems, San Jose, CA, USA/Ambion), and was then transcribed to cDNA using reverse transcriptase enzyme M-MLV (Promega). Quantitative real-time PCR was performed using PerfeCTa SYBR Green Fastmix (Promega). Primer sequences used for qRT-PCR are listed in Table S1.

Protein isolation and immunoblotting

Proteins were extracted from 10-d-old seedlings or tobacco leaves using the following buffer: 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM DTT, 1% IGEPAL CA-630, 1 mM Na₂MoO₄.2H₂O, 1 mM NaF, 1.5 mM Na₃VO₄, 1 mM EDTA, 1 mM PMSF, 10 mM antipain, 10 mM aprotinin, and 10 mM leupeptin. Samples were incubated for 1 h and centrifugated at 18 000 g and 4°C for 10 min. The supernatants were transferred to new 1.5 ml tubes, and the total protein concentration of each sample was measured using Bradford Reagent (Bio-Rad). An equivalent quantity of protein for each sample was loaded and separated by SDS-PAGE. Immunoblotting was performed using anti-GFP antibody.

Yariv staining and quantification of AGPs

After 10-d-old seedlings had been placed in half-strength MS (½MS) liquid medium for 24 h, they were placed in ½MS liquid medium with or without 100 mM NaCl. Roots from treated and untreated seedlings were immersed in 2 mM Yariv reagent for 1 h with shaking and were then washed three times with distilled water. The stained roots were examined and photographed using a Leica EZ4HD stereomicroscopy. AGPs were quantified as previously described (Gao et al., 1999; Schultz et al., 2000).

For the quantification of AGPs in growth medium, 12-d-old wild-type seedlings were transferred to ½MS liquid medium with or without NaCl (120 mM) and incubated for 6 h. After removing seedlings, the liquid media were filtered with two layers of Miracloth (475855-1R; Millipore) and centrifuged at 1000 g and 4°C for 90 min. After the samples cooled to room temperature, 0.5 ml of tert-butyl alcohol was added, and the samples were centrifuged at 15 min. The pellets were re-dissolved in 500 μl of 1% CaCl₂. Total carbohydrate content was determined using the phenol–sulfuric method (Dubois et al., 1956). β-d-Galactosyl–Yariv reagent was added with a final concentration of 1 : 45 of the total carbohydrate. The solutions were mixed and incubated at 4°C for 2 h, and then centrifuged at 10 000 g for 15 min. The pellets were washed twice using 1 ml 2% CaCl₂. Pellets were then dissolved in 500 μl of 20 mM NaOH, and absorbance at wavelength 457 nm was measured. Gum arabic in 1% (w/v) CaCl₂ in water was used as a standard with 1% CaCl₂ as a blank.

GUS staining

For histochemical GUS staining, MUR4-GUS, MEE25-GUS, DUR-GUS and MURL-GUS transgenic plants were incubated in a staining buffer (10 mM EDTA, 0.5 mM K₂Fe[CN]₅,100 mM sodium phosphate, pH 7.0, 5-bromo-4-chloro-3-indolyl β-d-glucuronide, 0.5 mM K₃Fe[CN]₅, and 0.1% Triton X-100) for 18 h. The stained samples were washed in 70% ethanol to remove all chlorophyll.

Cell integrity assay

Next, 6-d-old seedlings grown on ½MS medium were transferred to medium supplemented with 140 mM NaCl. After growth for 12 h, the roots were stained with propidium iodide (PI) before they were evaluated by confocal microscopy (Zeiss, Oberkochen, Germany). Cell death was defined as fully stained by PI. The number of burst cells in the elongation zone of roots was calculated and at least 10 roots for each genotype were analysed.
Split luciferase assay

*Agrobacterium* strains (GV3101) harbouring the corresponding constructs was grown in liquid Lysogeny Broth (LB) and harvested by centrifugation. The pellets were resuspended in an injection buffer (10 mM MgCl₂, 10 mM MES, pH 5.6, and 100 µM acetosyringone) with a final concentration of OD₆₀₀ = 0.5. The strains were incubated at room temperature for 2 h before infiltration. For co-infiltration, equal volume of two different strains carrying the indicated nLUC and cLUC constructs were mixed and infiltrated into *N. benthamiana* using a 1-ml disposable syringe. After 48 h, luciferin was sprayed on the infiltrated leaves, and the fluorescence was detected with a charge coupled device (CCD) camera (Princeton Instruments, Trenton, NJ, USA).

Cellular localisation and confocal microscopy

*A. tumefaciens* (GV3101) harbouring the *GmMan1-mCherry* plasmid was mixed with bacteria expressing 35S::MUR4–YFP, 35S::MURL–YFP, 35S::DUR–YFP, or 35S::MEE25–YFP. The mixed bacteria were coinfiltred into *N. benthamiana* leaves and YFP and mCherry signals were visualised after infiltration for 2 d by using confocal laser scanning microscopy.

**Results**

**MUR4 is required for root elongation under salt stress**

To identify genes that are required for salt stress tolerance, we generated an EMS-mutagenised mutant pool and screened for mutants that were hypersensitive to salt stress. One of the mutants, designated 20-2, was defective in root elongation under high-salinity conditions (Fig. S1a). By using whole-genome resequencing-based genetic mapping, a mutation in the *MUR4* gene was identified in the 20-2 mutant (Fig. S1b). To determine that *MUR4* was the mutated gene responsible for the salt-hypersensitive phenotype of the 20-2 mutant, we examined the phenotype of two published alleles of the *mur4* mutant, *mur4-1* and *hsr8-2* (Burget et al., 2003; Li et al., 2007), under high salt. Both mutant alleles showed normal root elongation on MS medium but much slower root elongation on MS medium supplemented with NaCl compared with the wild-type plants (Fig. 1a,b). However, the growth of the aerial part of *mur4-1 hsr8-2* mutants appeared to be unaffected by salt stress (Fig. 1a). These results indicated that MUR4 is required for root elongation under salt stress. Because the phenotypes of the *mur4-1* and *hsr8-2* mutant alleles were similar to that of the 20-2 mutant, we used these two mutant alleles for further study.

To further determine the salt-hypersensitive phenotype of the *mur4* mutant, we sowed the seeds of the wild-type and *mur4-1* on MS and NaCl media and assessed the rates of seed germination and cotyledon greening. The results showed that the rates of seed germination and cotyledon greening of the *mur4-1* mutant were normal on MS medium but were much slower than those of the wild-type on NaCl medium (Fig. 1c). The *mur4-1* mutants also exhibited slower root elongation than the wild-type on other salt media, such as KCl, NaNO₃, and KNO₃ media (Fig. 1d,e). To determine whether high salinity reduced the root elongation of the *mur4* mutant as a consequence of ion toxicity or osmotic stress, we transferred seedlings to MS medium containing a high concentration of mannitol, which can induce osmotic stress but not ion toxicity. The results showed that the roots of the *mur4-1 hsr8-2* mutants elongated normally on mannitol medium (Fig. 1a,b), indicating that the salt-hypersensitive phenotype of the *mur4-1 hsr8-2* mutants was caused by ion toxicity.

To confirm that the mutated *MUR4* was the genetic defect responsible for the salt-hypersensitive phenotype of the *mur4* mutant, the wild-type *MUR4* coding sequence driven by the CaMV 35S promoter was transformed into the *mur4-1* mutant. Two independent lines with high transcript levels of the *MUR4* gene were selected for phenotyping under salt stress (Fig. 1f). Both of the *MUR4* overexpression lines fully rescued the short root phenotype of the *mur4-1* mutant on NaCl and KCl media (Fig. 1g,h). Notably, the roots of the transgenic plants overexpressing the *MUR4* gene elongated slightly faster than those of the wild-type on NaCl medium (Fig. 1g,h).

The transcript level of *MUR4* is upregulated by NaCl and ABA

Because *MUR4* is important for salt tolerance, we tested whether salt stress may regulate the expression of the *MUR4* gene. After

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** MUR4 is required for root elongation under salt stress. (a) Here, 5-d-old Arabidopsis seedlings of the wild-type and two alleles of the *mur4* mutants, *mur4-1* and *hsr8-2*, were transferred to Murashige and Skoog (MS), MS+NaCl (100 mM), and MS+mannitol (200 mM) media, respectively. The seedlings were photographed 5 d after transfer. (b) Root lengths of the wild-type, *mur4-1*, and *hsr8-2* grown on MS, MS+NaCl (100 mM), and MS+mannitol (200 mM) media. Values are means ± SD (n = 10); *P < 0.05 (Student’s t-test). (c) Seed germination and cotyledon greening rates of the wild-type and *mur4-1* grown on MS and MS+NaCl (100 mM) media. Error bars indicate the SD of three biological replicates. (d) Here, 5-d-old seedlings of the wild-type and *mur4-1* were transferred to MS, MS+KCl (100 mM), MS+NaNO₃, (100 mM), and MS+KNO₃ (100 mM) media. The seedlings were photographed 5 d after transfer. (e) Root lengths of the wild-type and *mur4-1* seedlings grown on MS, MS+KCl (100 mM), MS+NaNO₃, (100 mM), and MS+KNO₃ (100 mM) media. Values are means ± SD (n = 10); *P < 0.05 (Student’s t-test). (f) qRT-PCR analysis of the transcript level of *MUR4* in the wild-type, *mur4-1*, and two independent *MUR4* overexpressing lines in the *mur4-1* mutant background (*mur4-1*MUR4OX1 and *mur4-1*MUR4OX2). ACTIN8 was used as the internal control. Values are means ± SD (n = 3); *P < 0.05 (Student’s t-test). (g) Phenotypes of the wild-type, *mur4-1*, and *MUR4* overexpressing plants grown on MS, MS+NaCl (100 mM), and MS+KCl (100 mM) media. (h) Root lengths of seedlings grown on MS, MS+NaCl (100 mM), and MS+KCl (100 mM) media. Values are means ± SD (n = 10); *P < 0.05 (Student’s t-test). (i) qRT-PCR analysis of the transcript level of *MUR4* in the wild-type plants after treatment with NaCl (150 mM) (i) or ABA (50 µM) (j). *ACTIN8* was used as the internal control. Values are means ± SD (n = 3); *P < 0.05 (Student’s t-test). (k) qRT-PCR analysis of the transcript level of *MUR4* in the wild-type, *aba2-1*, and *abi1-1* before and after NaCl (150 mM) treatment. Values are means ± SD (n = 3); *P < 0.05 (Student’s t-test). (l) *MUR4*-GFP transgenic plants were treated with NaCl (150 mM) for 0, 1, 3 or 6 h. Immunoblottings were performed using anti-GFP and anti-actin antibodies.
wild-type seedlings were treated with NaCl for 0, 1, 3, and 6 h. Quantitative real-time (qRT)-PCR analysis was performed. The result showed that the expression of \textit{MUR4} gene was upregulated after NaCl treatment (Fig. 1i). Similarly, treatment of wild-type seedlings with ABA also increased the expression of \textit{MUR4} gene (Fig. 1j), which prompted us to test whether the salt-upregulation expression of \textit{MUR4} gene expression depends on the ABA response pathway. To this end, the \textit{aba2-1} mutant, in which the
ABA biosynthesis is defective (González-Guzmán et al., 2002), and the abi1-1 mutant, in which the ABA core signaling pathway is repressed (Gosti et al., 1999), were treated with NaCl and the transcript level of MUR4 gene was examined in these two mutants. The upregulation of MUR4 expression by salt stress was attenuated in both aba2-1 and abi1-1 mutants (Fig. 1k). Consistent with the increased transcript level of MUR4 gene in the wild-type plants after salt treatment, the protein level of MUR4 was also increased after salt treatment (Fig. 1).

**Exogenous Ara rescues the salt-hypersensitive phenotype of the mur4 mutant**

Consistent with previous studies (Burget & Reiter, 1999; Li et al., 2007), our results showed that the Ara content in the cell walls of rosette leaves was reduced by c. 50% in both the mur4-1 and hsr8-2 mutant alleles, whereas transgenic plants overexpressing MUR4 had a higher Ara content than the wild-type plants (Fig. 2a). These results confirmed that MUR4 is required for the biosynthesis of UDP-Ara in Arabidopsis. We also examined the Ara content in 15-d-old seedlings before and after salt treatment, and found that salt treatment slightly reduced the Ara content in the wild-type, but substantially reduced the Ara content in the double mutants (Fig. 2b). To determine whether the reduced root elongation of mur4-1/hsr8-2 mutants under salt stress was caused by the reduced level of Ara, we transferred seedlings to NaCl medium supplemented with exogenous Ara. We found that the root elongation of mur4-1/hsr8-2 mutants on NaCl medium was restored to the wild-type level by exogenous Ara (Fig. 2c,d). By contrast, supplementation with two other monosaccharides, Glc and Xyl, failed to rescue the salt-hypersensitive phenotype of the mur4-1/hsr8-2 mutants (Fig. 2c,d). Together, these results indicated that Ara biosynthesis is important for maintaining root elongation under salt stress.

ARA1 and ARA2 catalyse the first step in returning Ara to the nucleotide sugar pool via a salvage pathway (Dolezal & Cobbett, 1991; Behmuller et al., 2016). To understand the role of the salvage pathway in salt stress tolerance, we assessed the phenotypes of ara1-2 ara2-1 and hsr8-2 ara1-2 ara2-1 triple mutants on NaCl medium. The ara1-2 ara2-1 double mutants showed a similar root length as the wild-type, and the hsr8-2 ara1-2 ara2-1 triple mutants exhibited a similar root length as the hsr8-2 single mutant under salt stress (Fig. 2e,f). Further study showed that exogenous Ara recovered the short root phenotype of the hsr8-2 single mutant, but not the short root phenotype of the mur4-1/hsr8-2 ara1-2 ara2-1 triple mutant under salt stress (Fig. 2e,f), which indicates that hsr8-2 plants required the ARA1/ARA2-mediated salvage pathway to be restored to wild-type by exogenous Ara.

Previous studies have shown that mutation of PRL1, MED25, or MED8 suppresses the sugar-hypersensitive hypocotyl elongation phenotype of the mur4-1/hsr8-1 mutant under dark conditions (Li et al., 2007; Seguela-Arnaud et al., 2015). Here, we examined the phenotype of med25 hsr8-1, med8 hsr8-1, and mur4-1 prl1-1 on salt medium, and found that the roots of these mutants were only slightly longer than those of mur4-1/hsr8-1 (Fig. S2a,b), indicating that MUR4/HSR8-mediated salt stress response pathway is distinct from the sugar-response pathway. Ara is involved in the decoration of side chains B and D of cell wall RG-II (Bar-Peled et al., 2012). Here, we found that application of boric acid, which is essential for the cross-linking of RG-II (O’Neill et al., 2001), partially suppressed the short root phenotype of the hsr8-2 mutant under salt stress (Fig. 2g), indicating that RG-II also contributes to root growth under salt stress.

**Cell–cell adhesion is disrupted in the mur4 mutant under salt stress**

Because the mur4 mutant showed abnormal root growth under salt stress, we used brightfield microscopy to examine the morphology of mur4-1 root cells before and after NaCl treatment. Without NaCl treatment, root cell morphology did not differ between the wild-type and mur4-1 mutant. Under salt stress, however, the wild-type plants were able to maintain straight root elongation, whereas the root cells of mur4-1 mutant were twisted and their cell–cell adhesion was severely disrupted (Fig. 3a). The 35S::MUR4 plants of the mur4 mutant had normal cell elongation and cell–cell adhesion (Fig. 3a). Using scanning electron microscopy, we confirmed that the root cells of the mur4-1 mutant under salt stress had abnormal shapes and were separated by large gaps (Fig. 3b). In many cases, the epidermal cells of the mur4-1 mutant were peeled away from the roots under high salinity (Fig. 3b). These results indicated that the Ara-containing components are critical for the maintenance of cell wall integrity and cell–cell adhesion under salt stress. In addition, we found that application of boric acid partially rescued the defective cell–cell adhesion of mur4-1 mutant under high salinity (Fig. S3a). Furthermore, we found that the elongation cell number in the roots of the mur4 mutant was not affected under normal conditions but was substantially reduced under high salinity compared with that in the wild-type (Fig. 3c). Plants with disrupted cell wall integrity showed increased cell bursting under high salinity.
Our result showed that the mur4 mutants exhibited more burst cells under high salinity compared with the wild-type (Figs 3d, S4), suggesting that arabinose-containing polymers are required for the maintenance of cell wall integrity under high salinity.
determine whether the reduced content of Ara in the mur4 mutant affected the formation of AGPs, we grew wild-type and mur4-1 seedlings on MS and NaCl media and stained the roots with Yariv reagent, which specifically binds to AGPs (Worden et al., 2012; Kitazawa et al., 2013). In the roots of the wild-type seedlings, the level of AGPs was slightly reduced by NaCl treatment (Fig. 4a). In the roots of the mur4-1 mutant, the level of AGPs was slightly reduced under normal condition, but was markedly decreased under high salinity compared with the wild-type (Fig. 4a). Quantification of AGPs using high-performance liquid chromatography (HPLC)–mass spectrometry also showed that the AGPs were substantially decreased in the mur4-1 mutants after NaCl treatment compared with the wild-type (Fig. 4b). To test whether AGPs are released from cells under high salinity, we measured AGP content in the growth medium after salt treatment. Our results indicated that high salinity accelerated the release of AGPs to growth medium in Arabidopsis (Fig. 4c). To understand whether the reduction of AGPs was the cause of the reduced root elongation of the mur4 mutant under salt stress, we examined the phenotype of mur4-1/hr8-2 mutants on NaCl medium supplemented with gum arabic, a commercial source of Acacia AGPs (Kitazawa et al., 2013). The results showed that the exogenous gum arabic restored the root elongation of mur4-1/hr8-2 mutants under salt stress to that of the wild-type level (Fig. 4d,e). Gum arabic also restored near wild-type level growth in the hsr8-2 ara1-2 ara2-1 triple mutant (Fig. 4f), which lacked the Ara salvage pathway, demonstrating that the restoration was by AGPs and rather than by Ara hydrolysed from the AGPs by endogenous cell wall enzymes. The disrupted cell–cell adhesion of the mur4-1 mutant under high salinity was also recovered when exogenous gum arabic was supplied (Fig. S3b).

Previous studies have shown that HPGT1, HPGT2, and HPGT3 are required for the galactosylation of AGPs and mutation of the genes encoding these three proteins greatly reduces AGP content (Ogawa-Ohnishi & Matsubayashi, 2015). To further determine that AGPs are required for root elongation under salt stress, we assessed the phenotype of the hpgt123 triple mutant grown on salt medium. Like the mur4 mutant, the hpgt123 mutant had normal root elongation on MS medium but showed reduced root elongation under salt stress (Fig. 4g,h). The defect in root elongation of the hpgt123 mutant under high salinity was...
Fig. 4 The salt-hypersensitive phenotype of the mur4 mutant is caused by the reduced levels of AGPs in Arabidopsis. (a) Roots of the wild-type, mur4-1, and MUR4 overexpressing plants (in the mur4-1 mutant background) that were treated or not treated with NaCl (100 mM) were stained with Yariv reagent. Roots were photographed with a light microscope. (b) Quantification of the content of AGPs in the roots of the wild-type and mur4-1 mutant before and after NaCl (100 mM) treatment. Values are means ± SD (n = 3); * P < 0.05 (Student’s t-test). (c) Here, 12-d-old wild-type seedlings were transferred to ½MS liquid medium with or without NaCl (120 mM). After growth for 6 h, the released AGPs in the growth media were measured. Values are means ± SD (n = 3); * P < 0.05 (Student’s t-test). (d) Phenotypes of the wild-type, hsr8-2, and mur4-1 grown on MS, MS+NaCl (100 mM), and MS+NaCl+gum arabic (1.5%) media. (e) Root lengths of the wild-type, hsr8-2 and mur4-1 seedlings grown on MS, MS+NaCl (100 mM), and MS+NaCl+gum arabic (1.5%) media. Values are means ± SD (n = 10); * P < 0.05 (Student’s t-test). (f) Phenotypes of the wild-type and hsr8-2 ara1-2 ara2-1 seedlings grown on MS, MS+NaCl (100 mM), and MS+NaCl+gum arabic (1.5%) media. (g) Phenotypes of the wild-type and hpgt1 hpgt2 hpgt3 (hpgt123) seedlings grown on MS, MS+NaCl (100 mM), and MS+NaCl+gum arabic (1.5%) media. (h) Root lengths of the wild-type and hpgt123 grown on MS, MS+NaCl (100 mM), and MS+NaCl+gum arabic (1.5%) media. Values are means ± SD (n = 10); * P < 0.05 (Student’s t-test).
rescued by the addition of exogenous gum arabic (Fig. 4g,h). These results indicated that AGPs are required for root elongation under salt stress, and that the decreased level of AGPs is the main cause of the salt-hypersensitive phenotype of the mur4-11 hsr8-2 mutants.

MUR4 forms either a homodimer or heterodimers with its isoforms

To identify proteins that are associated with MUR4 in the regulation of UDP-Ara biosynthesis, we performed immunoprecipitation—mass spectrometry (IP-MS) analysis using MUR4–GFP transgenic plants. Transgenic plants expressing free GFP were used as the control. The peptides that belonged to the DUR protein were identified in the IP-MS data generated from the MUR4–GFP sample, but not from the control sample (Table S2). The DUR gene encodes an isoform of MUR4 in Arabidopsis. The potential interaction of MUR4 with DUR suggests the possibility that MUR4 might form a homodimer with itself and heterodimers with its isoforms. By aligning the MUR4 protein sequence against the Arabidopsis database at The Arabidopsis Information Resource (TAIR), we identified another two isoforms of the MUR4 protein, MEE25 and AT4G20460. Because the protein sequence of AT4G20460 was similar to that of MUR4, we named this gene MURL (MUR4-like). The MUR4 protein shares 79% and 82% identity with DUR and MURL, respectively. According to the protein database at TAIR, the MEE25 gene encodes two protein isoforms, and the longer isoform of MEE25 shares 88% identity with MUR4. Split luciferase (split-LUC) assays indicated that MUR4 interacted with itself and also with DUR and MURL but not with MEE25 (Fig. 5a). The formation of the homodimer of MUR4 was also supported by co-immunoprecipitation (Co-IP) assays (Fig. 5b). Strikingly, none of these three isoforms could form homodimers or heterodimers with each other (Fig. S5).

Consistent with a previous study (Burget et al., 2003), we found that MUR4 was localised in the Golgi when it was transiently expressed in Nicotiana benthamiana or stably expressed in Arabidopsis (Fig. 5c,d). DUR and MURL were also localised in the Golgi (Fig. 5c). However, the GFP fluorescence of MEE25 did not overlap with but was near the fluorescence of the Golgi marker protein (Fig. 5c), suggesting that MEE25 is probably localised in the trans-Golgi network (TGN). The different subcellular localisations of MEE25 and MUR4 are consistent with the lack of interaction between these two proteins.

MUR4 and its isoforms redundantly regulate root elongation

Like MUR4, all the three paralogous genes were upregulated after NaCl and ABA treatment, and NaCl-induced expression of these three paralogous genes was alleviated in the aba2-1 and abi1-1 mutants (Fig. S6). Histochemical staining of plants transformed with MUR4pro:GUS, MURLpro:GUS, and DURpro:GUS revealed that MUR4, MURL, and DUR were expressed in roots, especially in the elongation zone, and were also expressed in leaves and flowers (Fig. S7a–c). MEE25 was highly expressed in flowers, but weakly expressed in roots and leaves (Fig. S7a–c). To investigate whether the three paralogues are also involved in salt stress tolerance, we assessed the salt sensitivity of the T-DNA insertion mutants of DUR, MURL, and MEE25 (Fig. S7d,e). The result showed that none of these was defective in root elongation under salt stress compared with the wild-type (Fig. S7f,g), suggesting that MUR4/HSR8 plays a major role in the regulation of root elongation under salt stress.

To investigate whether DUR, MURL, and MEE25 function redundantly with MUR4/HSR8 in the regulation of root elongation and whether these three isoforms also contribute to UDP-Ara biosynthesis, we crossed the hsr8-2 mutant allele with dur, murl, and mee25 to generate double, triple, and quadruple mutants. The root growth of the hsr8-2 dur, hsr8-2 murl, and hsr8-2 dur murl mee25 mutants on MS medium was similar to that of the wild-type (Fig. 6a,b), and the murl dur mee25 triple mutant showed slightly reduced root elongation on MS medium compared with the wild-type. However, the hsr8-2 murl double mutant exhibited substantially slower root elongation on MS medium, and the root elongation was further inhibited in the hsr8-2 murl mee25 and hsr8-2 murl dur triple mutants and in the hsr8-2 murl dur mee25 quadruple mutant (Fig. 6a,b). Under salt stress, the hsr8-2 dur and hsr8-2 murl mee25 double mutants displayed a similar root length as the hsr8-2 single mutant, while the root elongation was slightly reduced in the hsr8-2 murl, hsr8-2 dur mee25, and hsr8-2 murl mee25 mutants, but was substantially reduced in the hsr8-2 murl dur triple mutant compared with the hsr8-2 single mutant (Fig. 6a,b). For the hsr8-2 murl mee25 quadruple mutant, the root growth was almost completely arrested and the leaves were chlorotic under salt stress (Fig. 6a,b). These results showed that MUR4/HSR8 functions redundantly with its paralogous proteins, especially with MURL, in the regulation of root elongation, and also suggested that MUR4 and together with its three paralogous proteins are required for the regulation of salt stress tolerance in leaves. The short root phenotype of hsr8-2 murl, hsr8-2 murl dur, and hsr8-2 murl dur mee25 grown on MS medium was restored to that of the wild-type by adding Ara and was partially suppressed by adding gum arabic to the medium (Fig. 6c,d). The Ara content in the rosette leaves, we investigated whether the three isoforms of MUR4 contribute to the biosynthesis of the remaining Ara content. To answer this question, we measured the Ara content in the rosette leaves of single, double, triple, and quadruple mutants. While the Ara content was reduced by 50% in the hsr8-2 mutant, none of the dur, murl, and mee25 single mutants showed reduced Ara content (Fig. S7h). The dur murl mee25 triple mutant had a 14% reduction in Ara content compared with the wild-type (Fig. 6e), indicating that these three paralogues are
also involved in the biosynthesis of UDP-Ara. Unexpectedly, for hsr8-2 dur, hsr8-2 mee25, hsr8-2 murl, hsr8-2 dur mee25, hsr8-2 murl mee25, hsr8-2 murl dur, and hsr8-2 murl dur mee25 mutants, all of which included the hsr8-2 mutation background, their Ara contents were the same or only slightly reduced compared with that in the hsr8-2 single mutant (Fig. 6e). These results suggested that MUR4/HSR8 contributed to the majority of Ara biosynthesis in the rosette leaves.

Because the obvious phenotypic characteristic of the mur4-1/hsr8-2 single mutant and its higher order mutants were observed
in roots, we then measured the Ara content in roots. Unlike the 50% reduction of Ara content in the rosette leaves, the Ara content was reduced by only ca. 15% in the roots of the mur4-1/hsr8-2 mutants compared with the wild-type (Fig. 6f). Under salt stress, the Ara content was reduced by ca. 33% in the roots of the mur4-1/hsr8-2 mutants compared with that in the wild-type (Fig. 6f). Interestingly, the Ara content was substantially lower in the roots of the hsr8-2 murl double mutant than that in the roots of the hsr8-2 single mutant and was even lower in the roots of the hsr8-2 murl mee25 and hsr8-2 murl dur triple mutants (Fig. 6g). In the roots of hsr8-2 murl dur mee25 quadruple mutant, the Ara content was reduced by ca. 52% compared with the wild-type and
was reduced by c. 44% compared with the hsr8-2 single mutant (Fig. 6g). These results indicated that the isoforms of MUR4 are involved in the biosynthesis of UDP-Ara in roots, which is consistent with the short root phenotype of the higher order mutants under normal conditions.

**ARA1 and ARA2 contribute to salt stress tolerance**

Our study suggested that the isoforms of MUR4 also contributed to the UDP-Ara biosynthesis pathway (Fig. 7a). To further clarify the contribution of both *de novo* and salvage pathways of UDP-Ara biosynthesis to salt stress tolerance, we generated the hsr8-2 *murl dur mec25 ara1-2 ara2-1* sextuple mutant. The roots of the sextuple mutant were shorter than those of the hsr8-2 *murl dur mec25* quadruple mutant on both MS and NaCl media, and the leaves of the sextuple mutant were more chlorotic than that of the quadruple mutant on NaCl medium (Fig. 7b,c). These results suggested that ARA1 and ARA2 are also important for salt stress tolerance. The short root phenotype of the sextuple mutant on either MS or NaCl medium could not be recovered by exogenous Ara (Fig. 7b,c), further supporting that the ARA1/ARA2-mediated salvage pathway was required for the assimilation of exogenous Ara. Our results also showed that gum arabic partially suppressed the salt-hypersensitive phenotype of the sextuple mutant (Fig. 7d). Quantitative analysis of Ara content revealed that, relative to the hsr8 *murl dur mec25* quadruple mutant, the sextuple mutant had a similar Ara amount in its roots and a reduced Ara content in its rosette leaves (Fig. 7e,f).

**Discussion**

The biosynthesis of polysaccharides in the plant cell wall is dynamically regulated in response to a variety of environmental stresses. Our study showed that the Ara content in the cell wall was altered after salt treatment and the plants that were disrupted in Ara biosynthesis were hypersensitive to high salinity, including NaCl, KCl, NaNO₃, and KNO₃, which suggest that maintaining the arabinosylation of cell wall components is critical for plants to adapt to adverse environmental conditions. Even though some salts, such as KNO₃, are essential plant nutrients, high concentrations of these salts inhibited the growth of plants. Our study showed that the three paralogues of MUR4 also contributed to the biosynthesis of UDP-Ara, and provided new insights into the UDP-Ara biosynthesis network.

Ara is involved in the decoration of a number of polymers and glycoproteins in the cell wall (Ogawa-Ohnishi et al., 2013; Borassi et al., 2016; Kotake et al., 2016). Our results showed that application of gum arabic, a commercial product of AGPs derived from *A. senega* (Kitazawa et al., 2013), recovered the root growth of *murl*/*hsr8* mutant under salt stress, suggesting that the short root phenotype of the *murl*/*hsr8* mutant under salt stress was caused by the alteration in root AGPs due to the limited availability of UDP-Ara. AGPs are macromolecules that are typified by the decoration of the protein backbone with Gal and Ara (Seifert & Roberts, 2007; Ellis et al., 2010; Nguema-Ona et al., 2013), and carbohydrates account for 90–98% of the total weight of AGPs (Knoch et al., 2014; Ogawa-Ohnishi & Matsubayashi, 2015). AGPs are cell wall components that undergo rapid turnover (Showalter, 1993; Behmüller et al., 2016). This study and others have all shown that high salinity accelerates the release of AGPs to growth medium and it was proposed that AGPs could serve in Na⁺/Ca²⁺ exchange. (Lamport et al., 2006, 2014; Olmos et al., 2017). Based on these results, we hypothesised that turnover of AGPs under salt stress may reduce Na⁺ activity and therefore attenuate ion toxicity in plants. While AGPs are released under high salinity, new AGPs are secreted to the cell wall to maintain cell wall integrity and root elongation. Production of new AGP protein backbones, Ara, and Gal is coordinately regulated to generate functional AGPs before they are secreted into the cell wall (Showalter & Basu, 2016). Elevation of the transcript and protein levels of MUR4 in the wild-type under salt stress may counteract the reduction of AGPs. However, disruption of Ara biosynthesis in the *murl* mutant would affect the formation of functional AGPs, and finally inhibit root elongation under salt stress. These results suggested that the salt-hypersensitive phenotype of the *murl* mutant was due to the deficiency in the structure of cell wall, but not caused by the disrupted physiological and molecular responses within cells under high salinity. Cell bursting assay indicated that cell wall integrity was disrupted in the *murl* mutant under salt stress, and resulted in a swelling phenotype and subsequently cell death.

The role of AGPs in root cell elongation has been reported in several studies. Treatment of Arabidopsis seedlings with Yariv reagent, which specifically binds to AGPs, inhibited root elongation (Ding & Zhu, 1997). *UGE4/REB1*, which encodes a UDP-D-Glc 4-epimerase, is required for the biosynthesis of UDP-D-
Gal and subsequently for the galactosylation of AGPs. The uge4/reb1 mutant is characterised by reduced root elongation and root epidermal cell bulging (Andème-Onzighi et al., 2002; Nguema-Ona et al., 2006). Mutation of the MURI gene, which is required for the biosynthesis of l-fucose, affects the fucosylation of AGPs and leads to a decrease in root cell elongation (van Hengel &

![diagram](image-url)
Roberts, 2002). SOS5, an AGP-like protein, is required for root growth under salt stress (Shi et al., 2003). The role of AGP proteins in root elongation is also supported by our finding that mutation of HPGT genes that are required for the galactosylation of AGP proteins (Ogawa-Ohnishi & Matsubayashi, 2015) showed arrested root growth under salt stress. The mechanisms underlying the role of AGPs in root elongation are still largely unknown. AGPs are probably required for regulation of the orientation of root cell elongation (Nguema-Ona et al., 2012).

Although gum arabic partially restored the wild-type growth in mutants of MUR6, boric acid also increased normal root growth in these mutants, indicating that dimerisation of RG-II also contributed to growth under NaCl stress. Four complex glycan side chains clustered in this pectic polysaccharide form RG-II-boron diester through their respective asparagine residues (Caffall & Mohnen, 2009). The mur1 mutation compromises the apiose-containing side chain because a fucosyl residue critical for structure is missing, but addition of boric acid restores normal growth (O’Neill et al., 2001) and stem tensile strength (Ryden et al., 2003). Ara residues are also critical subtending sugars of RG-II, and the partial recovery of the wild-type phenotype by boric acid implicates RG-II in growth upon challenge with NaCl.

Our study showed that Ara content in the mur4 mutant was reduced by c. 50% in the leaves but by only 15% in the roots of the mur4 mutant. In the mutant of the 1-fucose biosynthesis gene MURI, the 1-fucose content was reduced by more than 98% in the cell walls of leaves but by only 60% in the cell walls of roots (van Hengel & Roberts, 2002). These results may suggest that, relative to leaves, more functionally redundant proteins contribute to the biosynthesis of these monosaccharides in the roots. Our results showed that the Ara content in the rosette leaves of the higher order mutants of HSR8 with its paralogues was similar to that in the hsr8-2 single mutant, but that the Ara content in the roots was gradually reduced in the double, triple and quadruple mutants compared with the hsr8-2 single mutant, suggesting that the three paralogous proteins of MUR4 may mainly contribute to the UDP-Ara biosynthesis in roots. Because Ara still exists in the hsr8-2 murl dur mec25 quadruple mutant and even in the hsr8-2 murl dur mec25 ara1-2 ara2-1 sextuple mutant, we speculated that an unknown pathway may also be involved in the biosynthesis of UDP-Ara.

Even though Ara content is reduced in the roots of mur4/hsr8 mutant, the root elongation of mur4/hsr8 was similar to that of the wild-type on MS medium. However, hsr8-2 murl, hsr8-2 murl dur, hsr8-2 murl mec25, and hsr8-2 murl dur mec25 mutants all showed reduced root elongation under normal conditions. Because these higher order mutants contain less Ara than the hsr8-2 single mutant in roots, we speculated that a minimal threshold of Ara content in the cell walls of the primary root is required to maintain normal root elongation, and that the remaining Ara content in the roots of the hsr8-2 single mutant is sufficient to maintain root elongation under normal conditions. However, a substantial reduction of Ara content in the mur4/hsr8 mutant under salt stress leads to the disruption of cell wall integrity and therefore affects root elongation.

Acknowledgements

We thank Rebecca Stevenson for technical assistance, Prof. Raimund Tenhaken for providing ara1-2 ara2-1 seeds, Prof. Yoshikatsu Matsubayashi for providing hpgt123 seeds, and Prof. Michael W. Bevan for providing med25 hsr8-1 and med8 hsr8-1 seeds. This work was supported by the Strategic Priority Research Program (Grant no. XDB27040101) of the Chinese Academy of Sciences, and monosaccharide analyses were supported by the US Department of Energy, Office of Science, Basic Energy Sciences (Grant no. DE-SC000997).

Author contributions

J-KZ, CZ and OZ, conceived and designed the experiments; CZ and OZ performed most of the experiments; FZ, CL, LZ and YET constructed some plasmids and performed genotyping; PZ, C-CH and WAT conducted proteomics assays; NCC assisted in the analysis of cell wall monosaccharides, and CZ and J-KZ wrote the manuscript with constructive input from all authors. CZ and OZ contributed equally to this work.

ORCID

Nicholas C. Carpita  https://orcid.org/0000-0003-0770-314X
Chuan-Chih Hsu  https://orcid.org/0000-0002-7100-1401
Omar Zayed  https://orcid.org/0000-0003-1388-1903
Ling Zhang  https://orcid.org/0000-0002-2708-1176
Chunzhao Zhao  https://orcid.org/0000-0003-0284-2095
Jian-Kang Zhu  https://orcid.org/0000-0001-5134-731X
Peipei Zhu  https://orcid.org/0000-0003-4508-022X

References


borate cross-linking of rhamnogalacturonan-II. *The Plant Journal* 96: 1036–1050.


**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Identification of 20-2 mutant that is defective in root elongation under salt stress.

**Fig. S2** Mutations in MED25, MED8, and PRL1 genes slightly increase the root length of the *mur4/hsr8* mutants under salt stress.

**Fig. S3** The disrupted cell–cell adhesion in the roots of the *mur4-1* mutant is rescued by applying boric acid and gum arabic.

**Fig. S4** *mur4* mutant shows more burst cells than wild-type under high salinity.

**Fig. S5** The isoforms of MUR4 do not form homodimers or heterodimers with each other.

**Fig. S6** Salt stress-induced upregulation of the three paralogous genes of *MUR4* depends on ABA-mediated signaling pathway.

**Fig. S7** Disruption of each paralogous gene of *MUR4* does not affect root elongation under salt stress.

**Table S1** Primers used in this study.

**Table S2** IP-MS data generated from transgenic plants expressing 35S::MUR4–GFP or 35S::GFP.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.