Ethylene inhibits rice root elongation in compacted soil via ABA- and auxin-mediated mechanisms

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Soil compaction represents a major agronomic challenge, inhibiting root elongation and impacting crop yields. Roots use ethylene to sense soil compaction as the restricted air space causes this gaseous signal to accumulate around root tips. Ethylene inhibits root elongation and promotes radial expansion in compacted soil, but its mechanistic basis remains unclear. Here, we report that ethylene promotes abscisic acid (ABA) biosynthesis and cortical cell radial expansion. Rice mutants of ABA biosynthetic genes had attenuated cortical cell radial expansion in compacted soil, leading to better penetration. Soil compaction-induced ethylene also up-regulates the auxin biosynthesis gene OsYUC8. Mutants lacking OsYUC8 are better able to penetrate compacted soil. The auxin influx transporter OsAUX1 is also required to mobilize auxin from the root tip to the elongation zone during a root compaction response. Moreover, osaux1 mutants penetrate compacted soil better than the wild-type roots and do not exhibit cortical cell radial expansion. We conclude that ethylene uses auxin and ABA as downstream signals to modify rice root cell elongation and radial expansion, causing root tips to swell and reducing their ability to penetrate compacted soil.

Significance

Intensive agriculture and changing tillage practices are causing soils to become increasingly compacted. Hard soils cause roots to accumulate the hormone ethylene, triggering reduced root elongation and increased radial swelling. We demonstrate that ethylene regulates these distinct root growth responses using different downstream signals, auxin, and abscisic acid (ABA). Auxin is primarily required to reduce cell elongation during a root compaction response, whereas ABA promotes radial cell expansion. Radial swelling was originally thought to aid root penetration in hard soil, yet rice ABA-deficient mutants disrupted in radial swelling of root tips penetrate compacted soil better than wild-type plants. The combined growth responses to auxin and ABA function to reduce the ability of roots to penetrate compacted soil.

roots | soil compaction | ethylene | auxin | ABA

Soil compaction represents a major challenge facing modern agriculture, reducing crop yields by ~25%, and when combined with drought by up to 75%, as roots are challenged to penetrate hard soils. Europe has over 33 million hectares of soil prone to compaction, which represents the highest in the world as compared to Asia (10 million hectares) and Africa (18 million hectares). Soil compaction inhibits root penetration and decreases uptake of water and nutrients. Efforts to mitigate the impacts of soil compaction on crop productivity include reducing tillage, controlled traffic farming and subsoil management. However, these approaches can be time consuming, costly to implement and ineffective for deeper soil profiles.

Breeding crops to better withstand compacted soil offers a genetic solution to improve root growth during mechanical impedance. Recently, maize roots featuring small cells with thick walls in outer cortical layers (termed multiseriate cortical sclerenchyma [MCS]) were reported to improve rooting depth in compacted soil in greenhouse and field trials. The MCS anatomical trait was associated with greater root lignin concentration, tensile strength and root tip bending force compared to non-MCS genotypes. Conventional thinking considers that roots are unable to penetrate compacted soils because axial root tip growth force exerted by the plant roots is less than mechanical resistance produced by compacted soil. However, we recently discovered that plant roots use the gaseous hormone ethylene to sense soil compaction. Our experiments revealed that compacted soil restricts diffusion of ethylene out of and away from roots, triggering inhibition of root elongation while also promoting radial swelling. Remarkably, roots of Arabidopsis and rice mutants that are insensitive to ethylene are able to better penetrate compacted soil. Ethylene-response mutant roots remain narrow and able to penetrate compacted soil, whereas wild-type roots exhibit inhibition of cell elongation and promotion of cortical cell radial expansion. How ethylene triggers these root growth changes is currently unclear.

Here, we reveal key roles for the hormone signals auxin and abscisic acid (ABA) and their biosynthesis pathways as regulatory targets of the ethylene-response pathway during rice root growth responses to soil compaction. Exogenous treatment with ABA promotes radial expansion of root cortical cells, whereas auxin inhibits root cell elongation. Furthermore, we demonstrate that ABA acts downstream of ethylene and auxin signaling during a soil compaction response. Integrating ethylene, auxin, and ABA responses during a soil compaction response appears to support ABA-mediated radial cell growth.
expansion. We conclude that ethylene uses auxin and ABA as downstream signals to modify rice root elongation and radial expansion, disrupting the root’s ability to penetrate compacted soil.

Results

Soil Compaction Induces ABA Biosynthetic Gene Expression and Hormone Levels. Several hormone signals have been reported to function downstream of ethylene (11–14) to inhibit root elongation, including auxin and ABA (15–17). However, the interaction of these hormones has not been studied in roots during growth in compacted soil. To address this point, we initially measured ABA levels in rice primary root tip tissues from plants grown in either noncompacted (1.1 g cm\(^{-3}\) bulk density [BD]) or compacted (1.6 g cm\(^{-3}\) BD) soil conditions using ultra-high performance liquid chromatography-electrospray tandem mass spectrometry (UHPLC-MS/MS). ABA levels increased threefold in root tips growing in compacted versus noncompacted soil conditions (Fig. 1A).

To reveal why root tips grown in compacted soil have increased ABA levels, we examined the effect of soil compaction on transcript abundances of nine ABA biosynthetic genes (CRTISO, MHZ5, ABA1, ABA2, MHZ4, AAO1, AAO2, NCED2 and ZEP) in root tip tissues (Fig. 1B). Transcript analysis revealed that the majority (seven out of nine) of the ABA biosynthetic genes profiled were up-regulated in wild-type root tips grown in compacted versus noncompacted soil conditions. In contrast, none of the ABA biosynthetic genes were up-regulated significantly in root tips of the ABA biosynthetic mutant mh5-1 grown in compacted soil conditions (SI Appendix, Fig. S1A). Expression of ABA biosynthesis genes has been reported to be subject to positive feedback regulation by ABA (18), suggesting that mutating MHZ5 may attenuate positive up-regulation of other biosynthesis genes by ABA.

ABA Biosynthesis Is Up-Regulated by Ethylene during a Root Compaction Response. To examine the regulatory relationship between ethylene and ABA during root responses to compaction,
we treated the wild-type (cv. Nipponbare) and ethylene-insensitive mutants osein1 and osein2 (19) with either ethylene or ABA. While roots of the osein1 and osein2 mutants did not respond to ethylene treatment, width of wild-type roots doubled largely due to radial expansion of cortical cells (SI Appendix, Fig. S2 A–G). In contrast, ABA treatment caused cortical cells of all three lines to undergo radial expansion, mimicking the effects of ethylene on the wild-type roots (see Fig. 1D versus control in Fig. 1C for wild-type images, plus mutant images in SI Appendix, Fig. S3; cortical cell diameters ± ABA treatments are shown in Fig. 1E). This observation suggested that ABA can trigger cortical cell radial expansion, and this signal acts downstream of the ethylene pathway in modulating root growth in response to soil compaction. To validate that ABA acts downstream of ethylene during a root compaction response, we examined the osein2 mhz5-1 double mutant and OsEIN2 OE mhz5-1 (OsEIN2 overexpressing line crossed with ABA biosynthetic mutant mhz5-I) in compacted and noncompacted soil conditions. Our results revealed that neither line exhibited cortical cell radial expansion in compacted soil (SI Appendix, Fig. S4 A–F), validating that ABA acts downstream of ethylene during a compaction response and is required for radial expansion of cortical cells.

To determine how ethylene regulates ABA levels, we examined the expression of ABA biosynthetic genes in wild-type rice root tip treated with ethylene. Ethylene was observed to elevate transcript levels of the ABA biosynthesis genes profiled (MHZ5, CRITISO and ZEP, SI Appendix, Fig. S5), consistent with when roots are exposed to soil compaction conditions and the enhanced ethylene response promoted ABA biosynthesis (Fig. 1B). To understand in which tissues ABA biosynthetic genes were up-regulated by ethylene during a compaction response, we performed in-situ hybridization experiments on MHZ4 and MHZ5. This revealed induction of ABA biosynthetic genes in response to compacted soil conditions in root vascular tissues (SI Appendix, Fig. S6 A–H).

ABA Promotes Radial Expansion of Root Cortical Cells under Compacted Conditions. To determine whether ABA is essential for radial expansion of root cortical cells in compacted soil conditions, we examined the phenotypes of several rice ABA biosynthetic mutants. MHZ5 encodes an ethylene-inducible ABA biosynthetic enzyme (SI Appendix, Fig. S5) which, when mutated, displays an ethylene-insensitive root growth phenotype (20). Wild-type and mhz5-1 lines were grown in either noncompacted (1.1 g cm⁻³ BD) or compacted (1.6 g cm⁻³ BD) soil conditions, then excavated roots were radially sectioned and confocal imaged (see Materials and Methods). In contrast to the wild-type (Fig. 2A and C), root diameter of mhz5-I did not significantly increase when grown in compacted soil (Fig. 2B and D). Cortical cell width of wild-type roots doubled under compacted soil conditions (Fig. 2C and E), which was blocked in mhz5-I roots (Fig. 2D and E). Nevertheless, external treatment with ABA in a hydroponic system induced radial expansion of cortical cells in mhz5-I (and wild-type) roots by more than two-fold (Fig. 2F–J), confirming that mhz5-I remains sensitive to ABA. We also confirmed that ABA promotes root radial expansion in soil conditions. Treatments using a series of ABA concentrations (0, 10, 50, and 100 μM ABA) to wild-type roots grown in noncompacted soil (SI Appendix, Fig. S7A) revealed that 10 μM ABA is sufficient to mimic soil compaction responses (SI Appendix, Fig. S7 C–F). Furthermore, mhz5-I remains sensitive to ABA in noncompacted soil conditions and exhibits a similar response (more than twofold increase in cortical cell diameter) as the wild-type roots (SI Appendix, Fig. S8 A–E). We conclude that ABA biosynthesis is required to promote root radial expansion in response to soil compaction conditions (Fig. 2).

ABA Biosynthetic Mutants Exhibit Increased Root Penetration in Compacted Soil. Next, we studied the impact of disrupting ABA biosynthesis on root penetration in compacted soil. To examine this, we performed noninvasive X-ray micro-computed tomography (CT) imaging (21) of wild-type and mhz5-I roots grown in noncompacted and compacted soil conditions (Fig. 3A–D). CT imaging in compacted soil revealed that mhz5-I root elongation was much less inhibited (~15% reduction) compared to wild-type (~40%) (Fig. 3K). Hence, cortical cell swelling in response to soil compaction does not aid root penetration capacity. Instead, ABA-dependent root swelling appears to reduce root elongation when exposed to compacted soil.

To independently validate this, we examined the root penetration ability of several other rice ABA biosynthetic mutants (Fig. 3 and SI Appendix, Fig. S1B) (22–24). The osaba1 mutant is unable to catalyze oxidation of zeaxanthin to produce trans-violaxanthin and has much less ABA than the wild-type plants (22), while the osaba2 mutant is unable to produce abscisic aldehyde that is converted to ABA by AAO (abscisic aldehyde oxidase) (23). The mh4 mutant, which also contains less ABA (61% of wild-type), is homologous to the Arabidopsis aba4 mutant (24). Arabidopsis ABA4 encodes neoxanthin synthase, which converts transviolaxanthin to neo-violaxanthin, and the aba4 mutant does not accumulate ABA under water-deficit stress (25). CT imaging revealed that all of the ABA biosynthetic mutants examined (osaba1, osaba2, mh4, and mh5-I) exhibited higher root penetration ability than wild-type roots in compacted soil (Fig. 3E–K). Hence, our mutant analysis confirmed that ABA functions to reduce root penetration in compacted soil conditions.

To probe the role of ABA in regulating root penetration activity, we quantified ABA levels in root tips of the ABA biosynthetic mutants grown in noncompacted (1.1 g cm⁻³ BD) and compacted soil (1.6 g cm⁻³ BD) conditions. UHPLC-MS/MS analysis revealed that, in contrast to wild-type, compacted soil conditions did not induce significantly higher ABA levels in the mutants (SI Appendix, Fig. S1C). ABA biosynthetic mutants also exhibited less radial expansion of cortical cells than the wild-type roots in compacted soil conditions (SI Appendix, Fig. S9 A–H). By comparing ABA accumulation (SI Appendix, Fig. S1C) and radial expansion (SI Appendix, Fig. S9 A–I) datasets, we conclude that higher ABA levels correlate with greater radial expansion of cortical cells (and reduction in root penetration ability) in compacted soil conditions.

Ethylene Inhibits Root Elongation during Soil Compaction via Auxin. Ethylene has been reported to inhibit root growth by elevating auxin levels in Arabidopsis thaliana (11, 13, 26), but the role of auxin during soil compaction conditions is unclear. To test whether and where auxin response is altered in rice roots grown in compacted soils, we imaged the roots expressing the DR5:VENUS auxin reporter (27, 28). Confocal imaging revealed that auxin response was significantly increased in root epidermal cells of wild-type (cv. Nipponbare) roots, specifically in the meristematic and elongation zones, when grown in compacted soil (1.6 g cm⁻³ BD) (Fig. 4, compare A to B for median plan view and C to D for maximum projection images). Although there was a slight reduction of auxin response in the root cap in compacted as compared to noncompacted conditions (Fig. 4H), there was a significant increase...
of auxin response specifically in expanding epidermal cells of roots grown in compacted soil (Fig. 4I). In contrast, when the DR5:Venus reporter was expressed in the ethylene-insensitive mutant osin2, auxin response was unchanged when grown in compacted compared to noncompacted soil conditions (Fig. 4, compare E to F for median plan view and G to H for maximum projection images, and Fig. 4J and SI Appendix, Fig. S10). Consistently, root elongation of the osin2 mutants was much less inhibited by soil compaction compared to the wild-type roots [Fig. 5A and C and ref. (10)]. Our observations suggest that auxin acts downstream of ethylene to mediate inhibition of root elongation during soil compaction.

To directly test the role for auxin under compacted soil conditions, we examined root elongation of wild-type (cv. Nipponbare) and ethylene-response mutants osel1 and osin2 in compacted soil with and without cotreatment with 1-naphthaleneacetic acid (NAA, a synthetic auxin analog). Consistent with auxin acting downstream of ethylene signaling, exogenous NAA application restored root elongation inhibition in osel1 and osin2 mutants under compacted soil conditions (Fig. 5B and C). However, external NAA application to osel1 and osin2 mutant roots in compacted soil did not induce radial swelling of cortical cells (<20%) as compared to ABA treatment (more than twofold) (SI Appendix, Fig. S11). Hence, ethylene inhibits root elongation via auxin during soil compaction stress.
Ethylene Up-Regulates Auxin Synthesis via OsYUC8 during Compaction Conditions. To determine how ethylene may up-regulate auxin during soil compaction stress, we functionally examined auxin-related targets of the primary ethylene response pathway. The ethylene-response transcription factor OsEIL1 has been shown to directly bind the promoter of the auxin biosynthesis gene OsYUC8 (SI Appendix, Fig. S12G) to regulate ethylene-mediated root elongation (29). Consistent with this previous report, root elongation of the loss-of-function mutant allele osyeuc8-2 was unresponsive to the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid) compared with its wild-type background (var. Hwayoung [HY]) (SI Appendix, Fig. S12 A–E). To reveal whether ethylene drives OsYUC8 up-regulation during root responses to soil compaction, OsYUC8 transcript abundance was quantified in wild-type, oseil1, and osein2 roots grown in compacted and noncompacted soils. OsYUC8 transcript levels were enhanced in wild-type roots grown in compacted soil (SI Appendix, Fig. S12F), but no difference is observed in osein2 and oseil1 roots between compacted and noncompacted conditions (SI Appendix, Fig. S12G). Hence, OsYUC8 expression was up-regulated in roots grown in compacted soil in an ethylene-dependent manner.

To functionally validate the role of OsYUC8 during the root compaction response, we phenotyped the loss-of-function mutant osyeuc8-2 versus its wild-type background (cv. Hwayoung). CT imaging revealed that osyeuc8-2 roots grown in compacted soil exhibited less inhibition of elongation than wild-type roots (Fig. S5D–H), which is similar to oseil1 lines (Fig. S5C), indicating that OsEIL1 is epistatic to OsYUC8. Hence, the ethylene (and OsEIL1)-regulated root response to compaction appears to rely on OsYUC8-mediated auxin biosynthesis.

Root Compaction Response Requires OsAUX1-Mediated Auxin Mobilization. OsEIL1 and OsYUC8 are mainly expressed in inner tissues of the root tip (10, 29), yet auxin response is elevated in the epidermis of roots grown in compacted soil (Fig. 4A–D). How does OsYUC8-derived auxin reach the epidermal cell layer? The Arabidopsis auxin influx carrier AtAUX1 plays a key role in ethylene-mediated inhibition of root elongation by facilitating auxin transport from the apex to the elongation zone (11, 13, 26). To assess the role of the orthologous OsAUX1 during rice root compaction responses, we characterized the osaux1-1 and osaux1-3 T-DNA insertion lines (30, 31). Roots of osaux1-3 were less sensitive to ACC than the wild-type (cv. Dongjin) (SI Appendix, Fig. S13 A and B), suggesting that OsAUX1 plays a key role in mediating ethylene-dependent inhibition of rice root elongation. CT imaging revealed that in contrast to the inhibition of root elongation in the wild-type, root lengths of both osaux1 mutant alleles were unchanged under compacted soil conditions (Fig. S5I–N and SI Appendix, Fig. S13C). Hence, the auxin influx carrier OsAUX1 appears essential for mobilizing auxin to its target root tissue(s) to mediate the inhibition of elongation in response to soil compaction conditions.

To reveal which root tissue(s) are targeted by auxin via OsAUX1 during the root compaction response, we analyzed...
compaction-induced changes in the spatial expression of the DR5:VENUS auxin reporter in wild-type and osaux1-3 backgrounds. Increased fluorescence intensity of the DR5:VENUS reporter in epidermal cells grown in compacted soil was not observed in osaux1-3 roots (SI Appendix, Fig. S14 A–E). Hence, much less shootward (i.e., root apex to base) auxin transport appears to occur in osaux1-3 versus wild-type roots, resulting in a reduced auxin response in epidermal cells of the meristematic and elongation zones (SI Appendix, Fig. S14 A–E). To understand the functional consequences of altered auxin response in this outermost root tissue, we measured epidermal cell lengths of wild-type and osaux1 roots grown in compacted and noncompacted soils. Epidermal cell lengths were significantly reduced in wild-type roots when grown in compacted soil, whereas the osaux1-1 and osaux1-3 lines exhibited much less difference between the compaction treatments (SI Appendix, Fig. S13 D and E). Hence, OsAUX1-dependent root compaction responses are associated with increasing shootward auxin transport to epidermal cells of the elongation zone, inhibiting cell and thus root elongation.

Does auxin also play a role in root radial expansion? No change was observed in osaux1 cortical cells in compacted soil (SI Appendix, Fig. S14 A–E).

Fig. 4. Soil compaction triggers higher auxin response in rice primary root epidermal cells to restrict epidermal cell expansion. (A–D) Representative confocal images (at median plane (A, B) and maximum projection (C, D)) of primary root tips of rice auxin reporter (DR5:VENUS) seedlings grown in (A, C) noncompacted (1.1 g cm\(^{-3}\)) and (B, D) compacted (1.6 g cm\(^{-3}\)) soil. (E–H) Representative confocal images (at median plane (E, F) and maximum projection (G, H)) of primary root tips of rice auxin reporter (DR5:VENUS) crossed with osein2 mutant seedlings grown in (E, G) noncompacted and (F, H) compacted soil. Scale bars in A–H represent 100 \(\mu m\). (I) Box plot showing the quantitative VENUS signal in the root cap and meristematic (MZ) plus elongation (EZ) zones of DR5:VENUS primary roots grown in 1.1 g cm\(^{-3}\) and 1.6 g cm\(^{-3}\) BD soil. (J) Box plot showing the quantitative VENUS signal in the root cap and meristematic (MZ) plus elongation (EZ) zones of the primary root in the osein2 mutant background grown in 1.1 g cm\(^{-3}\) BD and 1.6 g cm\(^{-3}\) BD soils. * and ** represent \(P\) values of ≤ 0.05 and 0.001, respectively, calculated by Student’s t-test.
Appendix, Fig. S15 A–C), consistent with the ethylene-mediated disruption in induction of key ABA biosynthesis genes (SI Appendix, Fig. S16). However, ABA treatment was able to promote radial expansion of cortical cells in osaux1 and osyuc8-2 mutant roots (SI Appendix, Figs. S17 and S18). Nevertheless, we noted that ABA did not fully restore osaux1 radial expansion to a wild-type level, suggesting that auxin (delivered via OsAUX1) may function to modify root epidermal properties to enable cortical expansion in response to ABA (SI Appendix, Fig. S17). In summary, we conclude that a compaction-induced ethylene
response uses auxin and ABA as downstream signals to modify rice root elongation and radial expansion, causing root tips to swell and reducing their ability to penetrate compacted soil.  

**Discussion**

Our results provide mechanistic insights into how rice roots adapt to soil compaction via ethylene-mediated up-regulation of auxin and ABA biosynthesis and movement. Reduced ethylene diffusion in compacted soil causes this signal to accumulate in root tip tissues (10), triggering the stabilization of OsEIL1, which then up-regulates expression of OsYUC8-mediated auxin biosynthesis. This newly synthesized auxin is transported by the auxin carrier OsAUX1 from its site of synthesis to its target site in epidermal cells of the elongation zone. The resulting higher auxin levels trigger inhibition of epidermal cell elongation, which acts to constrain cell elongation in inner root tissues, resulting in inhibition of root elongation. However, rice roots also employ ABA as another signal to promote cortical cell radial expansion in response to soil compaction (summarized in Fig. 6).

Increased synthesis of both auxin and ABA signals in response to compaction and elevated ethylene response causes wild-type root tips to swell. Classically, root tip swelling was reasoned to facilitate root penetration of compacted soil by widening the pores in the soil (8). However, we have experimentally demonstrated that root tip swelling is attenuated in rice ethylene- and ABA-mutants, yet they exhibit improved penetration abilities in compacted soil. This suggests that roots that remain narrower are better able to penetrate compacted soil. Conversely, roots that swell in compacted soil appear less able to penetrate.

Is there any evolutionary advantage for the ABA-induced root-tip swelling response during soil compaction? It is possible that in conditions of soil compaction where the root is growing into channels/cracks but otherwise cannot penetrate, root tip swelling might play the classically presumed role of widening the channels, thereby allowing root penetration that otherwise could not occur. Future work will explore the relationship between these hormone-driven root growth responses to compaction and structural features of the soil, such as the presence of channels and cracks, thickness of the compacted soil layer, and levels of moisture. Nevertheless, in our experimental system, where soil compaction is uniform, the root swelling response is not needed and in fact is negatively associated with penetration.
Soil compaction stress is also known to decrease water availability (32), which may serve to increase root ABA levels (in addition to the induction by ethylene). Although soil pores can be filled with water molecules in compacted soil conditions, the water is less accessible to roots as it is more tightly held (by greater matrix forces) in smaller pores in compacted soil than in larger pores in noncompacted soil, making it more difficult for plant roots to extract (32). Indeed, soil water release curves indicate lower water potential (moisture is less accessible to the plant) of compacted than noncompacted soils at the same soil water content (33). Thus, small void size and lower water potential render the water molecules less accessible for roots in compacted soils, which is likely to mimic mild drying conditions and therefore can enhance ABA levels in root tip tissues. Hence, promotion of radial swelling by ABA may serve as an adaptive response designed to increase root surface area and facilitate water uptake during compaction stress.

Materials and Methods

Soil Materials. A Newport series loamy sand (sand, 83.2%; silt, 4.7%; and clay, 12.1%; pH 6.35; organic matter 2.93%; FAO Brown Soil) was used in all soil experiments. Soil was collected from the University of Nottingham farm at Bunny, Nottinghamshire, UK (52.52°N, 10.7°W), passed through a <2 mm sieve and moisture content calculated by drying at 65 °C until constant weight.

Plant Materials and Growth Conditions. All rice plants for seed production were grown in paddy fields in Shanghai, China (30°N, 121°E) and Sanya, China (18°N, 109°E) in summer and winter, respectively. Seeds (Nipponbare, Hwayoung, Dongjin, DR5:VENUS, osaba1, osaba2, mhz4, mhz5-1, seo1, osein2, osein2, mhz5-1, EIN2 OE mhz5-1, osyuc8-2 (34), DR5:Venus/osaux1-3 and DR5:Venus/ossein2) were surface sterilized with 25% bleach for 10 min, then washed five times with autoclaved reverse osmosis (RO) water. The genetic backgrounds of the T-DNA insertion mutants are: osaba1, osaba2, mhz4, mhz5-1, seo1 and osein2, Nipponbare; osaux1-1 and osaux1-3, Dongjin; osyuc8-2, Hwayoung. Seeds were germinated on moist sterilized filter paper for 2 d in the dark at 28 °C in a growth chamber (12-h photoperiod at 300 μmol m⁻² s⁻¹ light intensity, 70% relative humidity).

After primary root emergence (4 to 6 mm in length), seedlings were gently placed in topsoil packed at different compaction levels (1.1 g cm⁻³) in a 3D printed column (33 mm diameter × 100 mm height), as previously described (10, 21). After 5 d, the primary root tips were carefully removed without washing the soil medium to keep the root tips intact for confocal imaging. Root lengths were analyzed via ImageJ (https://imagej.nih.gov/ij/).

Expression Profiling. Total RNA was isolated from wild-type (cv. Nipponbare) primary root tips (1 cm, encompassing the meristematic and elongation zones) of 7-d-old plants using TRizol reagent (Applied Biosystems) as per the manufacturer’s protocol. To remove genomic DNA contamination, isolated RNA was treated with DNase1 enzyme (1 U μg⁻¹ of total RNA). One microgram of total RNA from each of three independent biological replicates (each replicate comprised 10 to 15 root tips) was used for cDNA synthesis using ReverAid First-strand cDNA synthesis kit (Thermo Fisher) according to the manufacturer’s instructions.

SensiMix SYBR Hi-ROX (Bioline) master mix was used for qRT-PCR on a qTOWER real-time PCR System (Analytik Jena GmbH) with three biological replicates per treatment. Expression values were normalized with expression values of an endogenous control, ubiquitin5 (Os01g0328800). Relative expression levels (fold change: FC) were calculated using the 2⁻ΔΔCT method with respect to the control, as described previously (35). Primers used for rice ABA biosynthetic genes and their expression analysis are listed in SI Appendix, Table S1. All FCs were statistically evaluated using Student’s t test.

ABA Measurement from Rice Root Tips. For endogenous ABA quantification, wild-type (cv. Nipponbare) primary root tips (1 cm) were harvested from noncompacted and compacted soil-grown plants. The root tips were gently washed with autoclaved RO water to remove soil particles, immediately snap frozen in liquid nitrogen and stored at –80 °C. Extraction (8 to 10 mg fresh weight per sample) was performed according to (36); cold 10% methanol containing 1 M formic acid (vol/vol) was used as the extraction solvent. In brief, [²H₆]ABA was added as an internal standard prior to extraction, and samples were then extracted using 1 mL of extraction solvent, homogenized, and purified using Oasis HLB columns (30 mg/mL, Waters Corporation). For the data in Fig. 1A, ABA measurements were performed by HUPLC/MS/MS using an Agilent UPLC System (Waters) equipped with an Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm; Waters). For SI Appendix, Fig. S1C, analyses were conducted using a Phenomenex Polar C18 column (150 × 2.1 mm, 2.5 μm; Phenomenex), using MilliQ water (A) and ACN (B), both with 0.02% formic acid (vol/vol) as mobile phase, with measurements performed on a LC/MS/MS system comprising a 1260 Infinity II LC System coupled with 6495 Triple Quad LC/MS System, JetStream and Dual Ion Laser technologies (Agilent Technologies).

Radial Sectioning and Confocal Imaging. Primary roots were removed from soil columns and immediately washed with autoclaved RO water in three consecutive water-filled dishes using fine brush strokes to remove soil particles. Washing and clearing of the root tips were performed as previously described (21). One-centimeter root tips of auxin reporter lines were fixed in freshly prepared 4% PFA (Paraformaldehyde) and vacuum infiltration treatment was carried out for 2 h. Fixed root tips were washed with 1 × PBS buffer three times and kept in ClearSee solution for 24 h. Fluorescence of the cleared root tips was imaged with a TCS-SP8 confocal microscope using 10% argon and DPS5 lasers.

To obtain cross-sectional images, root tip samples (apical 1.5 cm) were embedded in 3.5% melted agarose and 100-μm-thick transverse sections were cut from the elongation zone using a Leica Vibratome, stained with Calcofluor White dye (Sigma) for 1 min on a glass slide, and imaged with a Leica SP5 confocal microscope using the UV laser.

X-Ray CT Imaging. Equally germinated seedlings (2 d of wild-type and osaba1, osaba2, mhz4, and mhz5-1 mutants were grown in 3D-printed columns (33 mm diameter × 100 mm height) filled with sandy loam soil at 1.1 g cm⁻³ and 1.6 g cm⁻³ BD for noncompacted and compacted soil conditions, respectively. The plants were grown in a growth chamber at 28 °C with a 12-h photoperiod at 300 μmol m⁻² s⁻¹ light conditions and 70% relative humidity. Five-day-old seedlings were scanned using a Phenix v (M 240 K X-ray CT system (Waygate Technologies [a Baker Hughes business]) at the Hounsfield Facility (University of Nottingham, Sutton Bonnington Campus, UK). The scans consisted of the collection of 2,520 projection images in FAST mode (continuous rotation), with an X-ray tube energy and current of 140 K and 200 μA, respectively. The detector exposure time was 131 μs, and the voxel resolution was 57 μm. Scan time was 5 min.

ABA Treatments in Hydroponic and Soil Systems. Five-day-old equally germinated seedlings (oselt1, oselt2, mhz5-1 mutants, and Nipponbare wild-type; oselt1-1, oselt1-3 mutants, and Dongjin wild-type) were transferred to a 2-L beaker containing autoclaved RO water and 10 μM ABA (Sigma) and grown for 2 d in a growth chamber at 28 °C with a 12-h photoperiod at 300 μmol m⁻² s⁻¹ PAR and 70% relative humidity. After 48 h, primary root tips (1 cm) were harvested, washed with RO water and placed in 4% PFA for 1 h. Subsequently, root tips were washed in PBS buffer two times and thereafter cleared in ClearSee solution for 15 d. Cleared root tips were then treated with Calcofluor White dye and imaged using confocal microscopy (Leica, TCS-SP5), as described previously (21).

Sandy loam soil was packed at 1.1 g cm⁻³ BD and the soil was treated with 25 mL of 0.1, 10, 50, and 100 μM ABA. Germinated seedlings were placed in the soil and grown in a growth chamber at 28 °C with a 12-h photoperiod at 300 μmol m⁻² s⁻¹ PAR and 70% relative humidity. The soil columns were treated with an additional 5 mL of the respective ABA concentrations on day 2, and on day 4 primary root tips (1 cm) were harvested, washed with RO water (five times), and fixed in a 4% melted agarose block for radial sectioning, staining with Calcofluor White dye, and imaging using TCS-SP5 confocal microscopy.

Ethylene Treatment. Germinated seedlings (oselt1, oselt2, mhz5-1 mutants, and Nipponbare wild-type; oselt1-1, oselt1-3 mutants, and Dongjin wild-type) were treated with 20 ppm ethylene gas as described previously (10). After 3 d of
Propidium Iodide Staining and Cell Length Analysis. Seeds were germinated in the dark at 28°C for 4 d, and germinated seedlings were grown in compacted and noncompacted soil for 4 d. Primary roots were harvested, cleaned with sterilized water, immersed in 1 μM propidium iodide for 3 min, and imaged using Leica Laser Scan Microscope (SP5) at an excitation wavelength of 561 nm.

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Data Availability. All study data are included in the article and/or supporting information.