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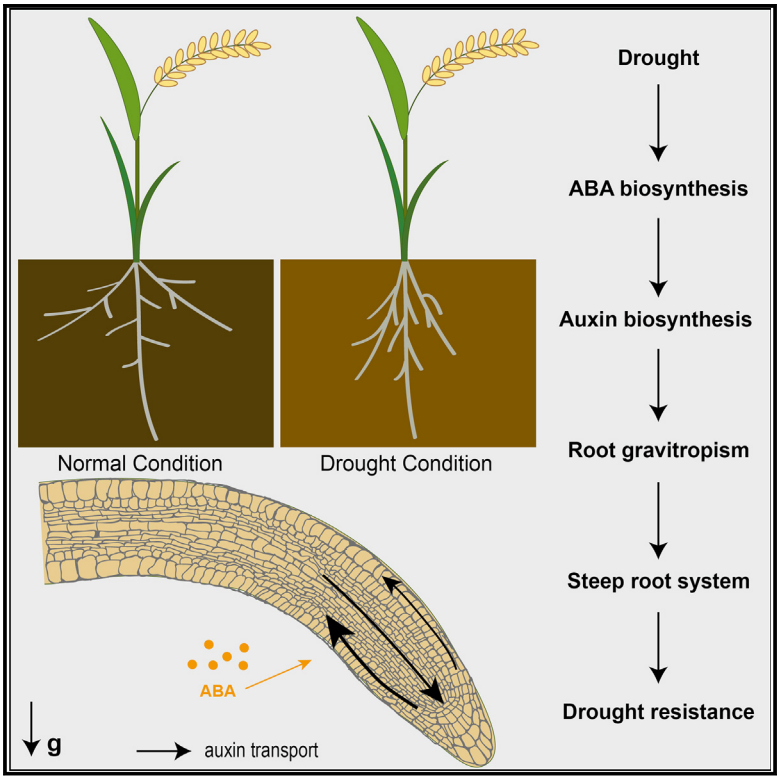
Current Biology

ABA-auxin cascade regulates crop root angle in response to drought

Graphical abstract



Highlights

- Crops shape shallow and steep RSA in normal and drought conditions, respectively
- ABA biosynthetic mutants exhibit a shallow RSA with no response to drought conditions
- ABA acts upstream of auxin biosynthesis signaling in root drought response

Authors

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In brief

Xiong et al. uncover a mechanism in rice and maize whereby ABA modulates auxin biosynthesis at the root tip, thereby controlling gravitropic machinery and root angle in response to drought conditions, suggesting the potential applicability of ABA for enhancing drought tolerance in cereal crops.

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ABA-auxin cascade regulates crop root angle in response to drought

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SUMMARY

Enhancing drought resistance through the manipulation of root system architecture (RSA) in crops represents a crucial strategy for addressing food insecurity challenges. Abscisic acid (ABA) plays important roles in drought tolerance; yet, its molecular mechanisms in regulating RSA, especially in cereal crops, remain unclear. In this study, we report a new mechanism whereby ABA mediates local auxin biosynthesis to regulate root gravitropic response, thereby controlling the alteration of RSA in response to drought in cereal crops. Under drought conditions, wild-type (WT) plants displayed a steep root angle compared with normal conditions, while ABA biosynthetic mutants (*mhz4*, *mhz5*, *osaba1*, and *osaba2*) showed a significantly shallower crown root angle. Gravitropic assays revealed that ABA biosynthetic mutants have reduced gravitropic responses compared with WT plants. Hormone profiling analysis indicated that the *mhz5* mutant has reduced auxin levels in root tips, and exogenous auxin (naphthaleneacetic acid [NAA]) application restored its root gravitropic defects. Consistently, auxin reporter analysis in *mhz5* showed a reduced auxin gradient formation in root epidermis during gravitropic bending response compared with WT plants. Furthermore, NAA, rather than ABA, was able to rescue the compromised gravitropic response in the auxin biosynthetic mutant *mhz10-1/tryptophan amino transferase2* (*ostar2*). Additionally, the maize ABA biosynthetic mutant *viviparous5* (*vp5*) also showed gravitropic defects and a shallower seminal root angle than WT plants, which were restored by external auxin treatment. Collectively, we suggest that ABA-induced auxin synthesis governs the root gravitropic machinery, thereby influencing root angle in rice, maize, and possibly other cereal crops.

INTRODUCTION

Food insecurity is a pressing international concern, especially as the world urbanizes.[1] Drought, a major abiotic stressor, has caused substantial crop production losses of approximately $30 billion over the past decade.[2] Climate change, particularly global warming, has further intensified drought conditions worldwide. With a projected population of 10 billion by 2050 and serious freshwater depletion, developing drought-resistant crops is of paramount importance.[3,4] The primary objective of this study is to examine the mechanisms underlying crop responses to drought conditions.

As sessile organisms, plants rely on their root systems, the primary organs for interacting with soil, to actively seek water. In drought conditions, water tends to evaporate from the topsoil and accumulate in the subsoil. The ability of roots to perceive the directions of water flow has long been recognized.[5] Consequently, the alterations in root system architecture (RSA) from shallow to steep rooting facilitate the extraction of water from deeper soil layers, thereby mitigating the effects of drought stress.[6–10] This shift toward steeper rooting is particularly advantageous in cereal crops such as maize (*Zea mays*) and rice (*Oryza sativa* L.), as it enables them to maintain high grain yields despite limited water availability.[7,8] Thus, breeding crop cultivars with a steeper root angle becomes crucial for ensuring yield stability and tackling global climate change.

RSA refers to the spatial distribution of roots in soil, influenced by several factors such as root length, number, and angle. In 1995, Digby and Firn introduced the concept of gravitropic setpoint angle (GSA). This is the angle between a root's growth direction and the gravity vector, with a vertically downward orientation being 0°.[11] The root angle is predominately influenced (or determined) by gravitropism.[10] Gravity, a key environmental factor, plays a crucial role in directing roots to grow in a downward direction. The process of root gravitropism is composed of several steps, including the perception of gravity within the columella cells via amyloplast sedimentation, signal transduction, and the establishment of asymmetric auxin distribution in the elongation zone. This ultimately leads to asymmetric cell

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elongation in upper and lower sides of the root, resulting in the root bending downward.[12]

Many important auxin-related genes/signaling pathways involved in root gravitropism have been identified in cereal crops. VILLIN2 (VLN2), an actin-binding protein in rice, governs root gravitropic responses by modulating microfilament dynamics, recycling PIN-FORMED2 (PIN2), an auxin efflux carrier, and subsequently affecting polar auxin transport.[13] Another actin-binding protein, RICE MORPHOLOGY DETERMINANT (RMD), regulates root angles by fine-tuning actin filament dynamics wrapped around the amyloplasts in root columella cells, thereby controlling amyloplast sedimentation.[14] Additionally, OsPIN2 and the auxin influx carrier AUXIN1 (OsAUX1) play roles in regulating root gravitropic responses by facilitating basal auxin transport in the root tip.[15,16] DEEPER ROOTING 1 (DRO1) and its homolog quantitative trait locus for SOIL SURFACE ROOTING 1 (qSOR1) are key players in root gravitropic growth in rice, operating downstream of auxin signaling.[7,17] SOR1/Mao huzi 2 (MHZ2), a RING finger E3 ubiquitin ligase, has been shown to control root gravitropism, demonstrated by the phenotype of its knockout mutant showing soil surface rooting.[18,19] Further, auxin promotes SOR1/MHZ2-dependent degradation of OsIAA26, which is an atypical Aux/indole acetic acid (IAA) protein to modulate ethylene inhibition of root growth in rice seedlings.[19] In maize, two auxin-related genes, *ZmRSA3.1* and *ZmRSA3.2*, contribute to regulating root angle and depth.[20] Recent studies have demonstrated that ethylene, an important gaseous hormone, plays a pivotal role in controlling root growth angle in rice and maize through the stimulation of auxin biosynthesis.[21]

Abscisic acid (ABA) is widely recognized as a critical modulator in plant responses to various environmental stimuli, including drought.[22] Its core signaling pathway comprises ABA receptors known as PYRABACTIN RESISTANCE/PYRABACTIN RESISTANCE-LIKE/REGULATORY COMPONENT OF ABA RECEPTORS (PYR/PYL/RCARs), CLADE-A TYPE-2C PROTEIN PHOSPHATASES (PP2Cs), and SUCROSE NONFERMENTING (SNF1)-RELATED PROTEIN KINASE 2 (SnRK2) kinases.[23] Under stress, ABA binds to its receptors to inhibit the activity of PP2C and releases the suppression of SnRK2s, resulting in the phosphorylation of downstream substrates.[23] Previous studies have indicated that ABA is a key regulator in the regulation of stomatal closure during drought response.[24,25] Additionally, ABA signaling is necessary for root hydrotropism in *Arabidopsis*.[26–28] Mutants defective in the core ABA signal transduction pathway—such as *abi2-1*, the *snrk2.2/2.3* double mutant, and *112458*, a sextuple PYR/PYL mutant—exhibit abnormal hydrotropic responses.[26,27] Furthermore, *MIZU-KUSSEI1* (*MIZ1*), a critical gene in the early phases of the hydrotropic response, is upregulated by ABA.[29,30] The antagonistic relationship between hydro-stimuli and gravi-stimuli suggests that ABA may exert a negative effect on root gravitropism in an auxin-independent manner.[28,31–34] However, water always accumulates in the deep soil layers under drought conditions and auxin signaling is needed for root gravitropism. Therefore, the impact of ABA on auxin-mediated root gravitropism and even RSA in response to drought stress, particularly in cereal crops, remains to be elucidated.

In this study, we report that ABA plays a crucial role in regulating root angle by mediating auxin biosynthesis in response to drought stress in both rice and maize. Through observation of RSA, it was showed that the root system of wild-type (WT) plants exhibited a steeper angle under drought compared with normal conditions. Hormone profiling analysis showed that drought stress induced ABA production, which in turn promoted root gravitropism, leading to a steeper root growth. ABA biosynthetic mutants displayed reduced root gravitropism and a shallower RSA with a weaker response to drought. Furthermore, these mutants showed lower auxin levels and responses. Notably, exogenous naphthaleneacetic acid (NAA) application restored root gravitropic defects in the mutants. Additionally, the auxin biosynthetic mutant (*mhz10-1*) displayed reduced root gravitropism and lacked a response to external ABA application. Our findings further confirm the conserved mechanism of the ABA-auxin biosynthesis module in regulating root angle in maize.

RESULTS

Rice forms a steep RSA under drought stress

Plants have evolved sophisticated mechanisms to adjust their RSAs in response to environmental conditions, such as drought, a common abiotic stress that causes serious loss in crop yield. Understanding how plants acclimate to drought by changing their RSA to access water from deep soil layers is crucial, as this has been shown to help plants survive water scarcity.[7–9] Our soil rhizotron assays showed that WT (cv. Nipponbare [Nip]) roots grown under drought conditions exhibited a steeper RSA with steeper root angles compared with those grown under normal conditions (Figures 1A–1C). Furthermore, this phenotype can be mimicked in paper roll semi-hydroponic systems by reducing water potential through the application of exogenous polyethylene glycol (PEG) treatment (Figures 1D and 1E). The rapid evaporation of water in the topsoil and its retention in deeper soil likely promoted rice plants to adjust their root systems transition from "shallow" to "steep" in response to water-limited conditions for survival. However, the molecular mechanisms of this adaptation remain unknown.

ABA biosynthetic mutants exhibit shallow RSA in drought conditions

ABA is a vital drought response phytohormone, with elevated levels observed in drought conditions.[22,35] Consistently, our hormone profiling measurements identified that ABA levels were induced by PEG treatments in the WT root tips compared with the normal conditions (Figure 2A). In order to ascertain the potential impact of ABA on RSA under drought, we conducted a comparative analysis of RSA in several ABA biosynthetic mutants (*mhz4*, *mhz5*, *osaba1*, and *osaba2*) and WT plants. MHZ4 is a homolog of AtABA4 in *Arabidopsis*, which is a neoxanthin synthase responsible for a branch of ABA biosynthesis.[36] *MHZ5* encodes a carotenoid isomerase indispensable for ABA biosynthesis.[37] The *osaba1* mutant is impaired in the epoxidation of zeaxanthin and has low ABA levels upon drought treatment,[38] and OsABA2 is a short-chain alcohol dehydrogenase critical in the last two steps of ABA biosynthesis.[39] WT plants showed a steeper root system in drought conditions compared with a shallower root system under normal conditions (Figures 2B and 2C). Intriguingly, all ABA biosynthetic mutants showed normal root

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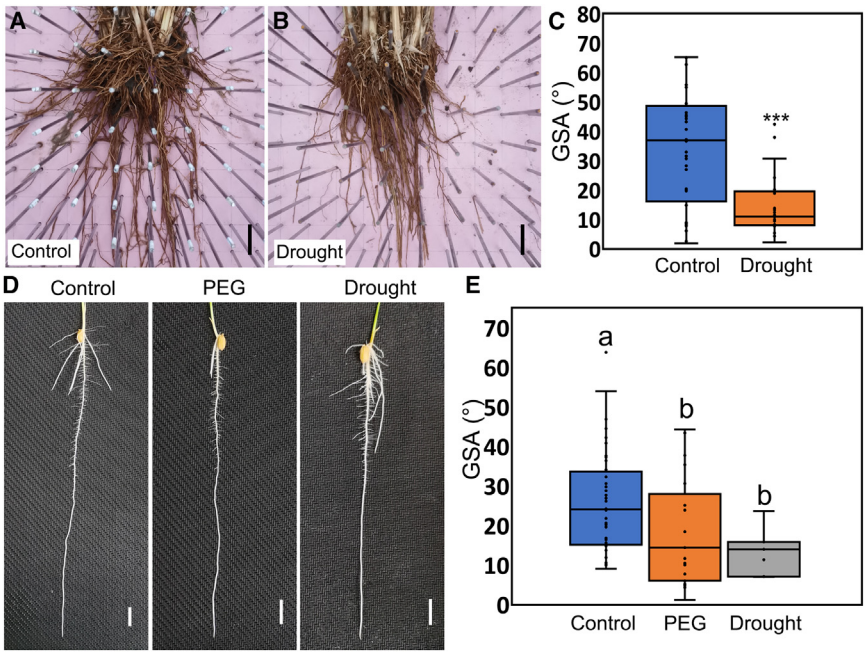

Figure 1. WT formed a steeper root system under drought stress

(A and B) Representative images showing root systems of WT (Nip) under control (A) and drought treatment (B) at reproductive stage observed using soil monolith approach. Scale bars, 5 cm.

(C) GSA analysis of crown roots of WT under control and drought treatments during the reproductive stage. We measured 3 individual plants. **indicates significant differences (Student's t test: ***$p < 0.001$, $n > 15$, n represents the total number of crown roots).

(D) Representative images showing root systems of 6-day-old seedlings of WT under control, 10% PEG, and drought treatments. Scale bars, 1 cm.

(E) GSA analysis of 6-day-old seedlings of WT under control, 10% PEG, and drought treatment. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of crown roots).

See also Figures S1 and S2.

number but a shallower RSA with no response to drought conditions compared with WT plants (Figures 2B–2D and S1). Moreover, this phenomenon was confirmed in soil non-invasively using X-ray micro-computed tomography (CT) (Figures 2E and 2F), indicating the necessity of ABA for the steeper root angle phenotype under drought conditions. Intriguingly, the RSA of *mhz5* in soil was slightly steeper under drought conditions than under normal conditions (Figures 2E and 2F), implying that ethylene is also involved in this process as drought induces ethylene overproduction[40] and ethylene could promote root gravitropism.[21] To validate this observation, ABA was exogenously applied to both WT and *mhz5* mutants. As expected, ABA treatment resulted in a steeper root system in WT plants and rescued the root angle phenotype defect observed in the mutants (Figures 2B, 2C, and S2). Collectively, these results indicate that ABA facilitates the formation of steep root systems under drought stress.

ABA promotes root gravitropism in rice

Root angle is considered to be regulated by competing gravitropic and anti-gravitropic offset mechanisms.[10] To determine which mechanism is affected in the regulation of ABA-mediated root angle, we performed root gravistimulation assays. Previous studies have demonstrated that varying concentrations of exogenous ABA have differential effects on root growth, with low concentrations favoring growth maintenance and higher concentrations restricting growth.[41,42] Additionally, root gravitropism is influenced by the rate of root elongation. To address this, we exposed WT roots to different concentrations of ABA and found that concentrations equal to or less than 100 nM did not impede the root growth rate and enhanced the root gravitropic growth angle (Figure S3). Higher levels of ABA (1 μM) inhibited root elongation and still caused quicker gravitropism (Figures S3 and S4). These findings indicate that ABA promotes the root gravitropic response regardless of root growth rate.

To further validate the promotional role of ABA in root gravitropism, we examined all ABA biosynthetic mutants (*mhz4*, *mhz5*, *osaba1*, and *osaba2*) and found that their attenuated gravitropic response in primary as well as crown roots could be effectively compensated by ABA treatment (Figures 3A–3C and S5), consistent with the shallow RSA exhibited by ABA biosynthetic mutants under water-deficient conditions (Figures 2B–2F). These observations demonstrate that ABA plays a positive role in root gravitropism, which may be necessary for the development of steep RSA under drought conditions.

ABA acts upstream of auxin response in regulating root gravitropism and, subsequently, root angle

Auxin is well known to regulate root gravitropism.[12] Root bending in gravitropism is attributed to differential cell elongation, determined by the asymmetric auxin distribution on the opposite sides of the root.[10,12] Additionally, research has shown that auxin levels in the root tip are modulated by ABA in rice.[43] To study the relationship between ABA and auxin in response to drought conditions, we conducted a comparative analysis of auxin levels in the root tips of WT and *mhz5* plants cultured with PEG treatments. The results consistently showed that auxin levels, similar to ABA, were induced in roots under PEG conditions (Figure 4A). In contrast, ABA biosynthetic mutants inherently exhibited lower auxin levels, which were not induced by PEG treatments (Figure 4A). Moreover, RT-qPCR analysis revealed that the expression of auxin biosynthetic genes *OsYUCCA8* (*OsYUC8*) and *MHZ10* in *mhz5* roots was significantly downregulated compared with the WT plants (Figures 4B and 4C). Additionally, reporter lines showed that ABA and PEG treatment induced the expression of *OsYUC8* and *MHZ10* in both primary roots and crown roots (Figures 4D–4G). These results suggested that ABA plays a regulatory role in the modulation of auxin biosynthesis in response to drought stress.

Auxin response reporter (DR5-VENUS) imaging revealed that auxin response in the lower and upper sides, and the root cap

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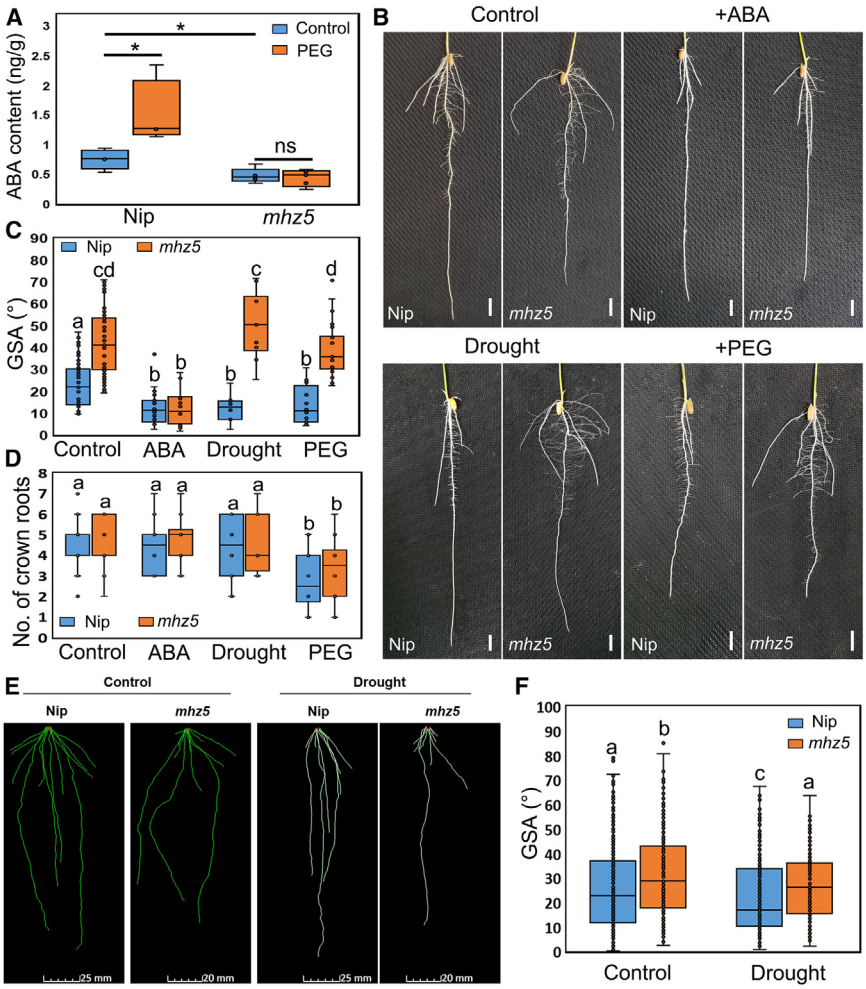

Figure 2. ABA treatment promotes the formation of steep root systems

(A) Measured ABA levels in primary root tips of WT and *mhz5* seedlings under 10% PEG treatment. **indicates significant differences (Student's t test: *$p < 0.05$; ns, not significant. $n = 4$, n represents the total number of biological replicates).

(B) Representative images showing root systems of 6-day-old seedlings of Nip and *mhz5* under control, 100 nM ABA, 10% PEG, and drought treatments. Scale bars, 1 cm.

(C) GSA analysis of 6-day-old seedlings of Nip and *mhz5* under control, 100 nM ABA, 10% PEG, and drought treatments. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of crown roots).

(D) Number of crown roots of 6-day-old seedlings of Nip and *mhz5* under control, 100 nM ABA, 10% PEG, and drought treatments. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 17$, n represents the total number of plants).

(E) Representative images showing root systems of Nip and *mhz5* under control and drought conditions via CT imaging.

(F) GSA analysis of Nip and *mhz5* under control and drought conditions. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 150$). We measured 4 individual plants. Each root was segmented into sections of 5 mm. n represents the total number of sections across all roots. A higher angle represents shallow root, while a lower angle represents steeper root growth.

See also Figures S1 and S2.

of the primary and crown root of *mhz5*, was notably weaker than that in WT under normal conditions (Figure 5). Consistent with the root gravitropism phenotype, PEG induced the auxin response in WT but not in *mhz5* (Figure 5). Furthermore, the ratio of auxin response between the lower and upper sides of the primary and crown roots in *mhz5* was lower than in WT under gravitropic response in both normal and simulated drought conditions, and ABA treatment restored the weak auxin response and the reduced ratio in *mhz5* roots (Figures 5I and 5S). Intriguingly, auxin response was much lower in crown roots compared with primary roots (Figures 5A–5T). These results further suggest that the accumulated ABA plays a role in modulating auxin biosynthesis that is crucial for facilitating root gravitropic bending and root angle, particularly under water limitation conditions.

To further confirm this hypothesis, exogenous NAA was applied to WT and *mhz5* mutant roots. Gravitropic defects of primary and crown roots observed in ABA biosynthetic mutants (*mhz5*, *osaba1*, and *osaba2*) were fully compensated by the NAA treatment (Figures 4H–4J). As expected, GSA of *mhz5* was effectively rescued by NAA treatments in comparison with WT plants (Figure S6). Similarly, NAA treatments restored the defective gravitropic responses of the auxin biosynthetic mutant *mhz10-1* and the auxin-transport-defective mutant *osaux1-3*, which also showed no response to ABA treatments (Figure S7). Overall, our findings suggest that during drought stress, increased ABA levels modulate auxin biosynthesis in the root tip. This, in turn, enhances the gravitropic response in emerging roots, leading to the formation of a steeper root angle.

During the soil compaction response, ABA suppresses root elongation and promotes root swelling through an auxin-biosynthesis-mediated process in rice.[43] The transcription factor basic region and leucine zipper 46 (OsbZIP46), involved in ABA signaling, can directly bind to the *OsYUC8* promoter to activate its expression in root growth.[43] However, our gravitropic treatment on the loss-of-function mutants *osbzip46-1* and *osbzip46-2* showed no significant difference compared with the WT (Figure S8). In consideration of the close relationship between OsbZIP46 and OsbZIP23,[44] we also analyzed the gravitropic response of *OsbZIP23* knockout mutant *osbzip23* and found no significant difference, even in the double loss-of-function mutant *osbzip23 osbzip46* (Figure S8). Additionally, ABI3 and ABI5 are two key components in ABA signaling responsible for regulating leaf angle and seed germination,[45–47] with their mutants exhibiting unaltered gravitropism (Figure S9). Consequently, we speculated that further investigation involving

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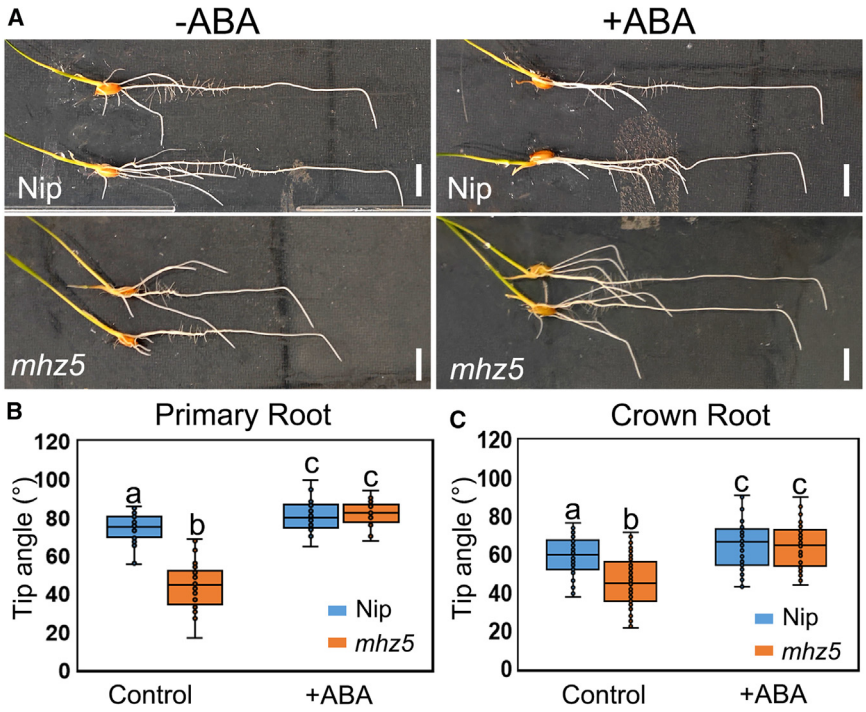

Figure 3. ABA plays positive roles in root gravitropism

(A) Representative images of roots of 4-day-old seedlings of Nip and *mhz5*, with or without 100 nM ABA treatment after 12 h gravistimulation. Scale bars, 1 cm.

(B and C) Tip angle analysis of Nip, *mhz5* primary (B), and crown (C) roots after 12 h gravistimulation, grown with or without 100 nM ABA treatment. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of roots).

See also Figures S3–S5.

additional transcription factors or higher-order mutants is warranted to elucidate this phenomenon.

The mechanism is conserved in maize

Given the distinct roles of ABA in root gravitropism in *Arabidopsis* and rice, it is imperative to investigate whether this mechanism of ABA-auxin biosynthesis module is conserved in other cereal crops, such as maize. We investigated the gravitropic response of ABA biosynthetic mutant *viviparous5* (*vp5*)[48] and WT maize plants. Our findings revealed a root gravitropic defect in *vp5* compared with WT plants (Figures 6A–6G). Additionally, *vp5* exhibited a shallower RSA (Figures 6H–6N). These results indicated the positive role of ABA in root gravitropism in maize.

To test the involvement of the ABA-auxin biosynthesis module in controlling maize root angle, exogenous NAA was applied to both WT and *vp5* roots. Mirroring the observations in rice, NAA treatments not only rescued the gravitropic defects but also the GSA of *vp5* (Figures 6A–6N). These results suggested that the mechanism of ABA-induced auxin biosynthesis promoting root gravitropic response under drought conditions is conserved in rice and maize.

DISCUSSION

The improvement of drought resistance in crops remains a significant global challenge due to the complex and not fully understood molecular mechanism. In this study, we propose a novel ABA-auxin-mediated pathway regulating root angle for improved drought tolerance in rice and maize (Figures 6O and 6P). Specially, elevated ABA levels under drought conditions upregulate auxin biosynthesis in the root tip, resulting in steeper root angle that enhances access to water from deeper soil layers.

Root angle is a key determinant of RSA in cereal crops, and steep rooting contributes to the maintenance of high grain yield in drought-prone areas.[6–10] Introducing DRO1 into a rice cultivar with a shallow root system induces a steeper root system and this facilitated higher yield with access to water in deep soil layers compared with the recipient cultivar under drought stress.[7] Despite the significance of root angle in crop drought resistance, the underlying molecular mechanisms remain largely unexplored, especially in cereal crops. Previous studies in *Arabidopsis* demonstrated auxin (rather than ABA) as a key regulator in regulating root gravitropism, whereas ABA (instead of auxin) signaling is indispensable for root hydrotropism.[26–28] Although these studies suggest an antagonistic relationship between ABA and auxin, our findings reveal a positive role of ABA in regulating root angle by modulating auxin biosynthesis in rice and maize. *Arabidopsis*, a dicot with a tap root system, contrasts with rice and maize, which are monocots with fibrous root systems. This suggests a possible diverse evolutionary pathway in monocots and dicots.

The intricate interplay between ABA and auxin in root growth and development has been extensively studied. Auxin has been suggested to act upstream of ABA, with ABI1, a key negative regulator of ABA signaling, being essential for auxin-modulated root development in *Arabidopsis*.[49] Additionally, AUXIN RESPONSE FACTOR 2 (ARF2), a transcription factor involved in auxin response, is reported to function as a negative regulator of ABA responses in seed germination and primary root growth.[50] However, further research in *Arabidopsis* has shown that ABA can also influence auxin signaling by reducing the expression levels of the PINs auxin efflux carriers, inducing the degradation of PIN2, decreasing auxin transport, and suppressing auxin responses to inhibit root elongation.[41,49,51–53] However, under moderate water stress, increased ABA accumulation modulates auxin transport in the root tip to sustain root growth in *Arabidopsis*, rice, and *Populus*.[35,54] This highlights the complex interplay between ABA and auxin, where their interaction can be antagonistic or cooperative, depending on the developmental stage and environmental cues.

In rice, ABA signaling transcription factor OsbZIP46 activates the expression of an auxin biosynthetic gene *OsYUC8*/*REIN7* to inhibit root elongation and promote root swelling.[43] However, neither the *osbzip46* mutants nor *osyuc8-2* mutant (a transfer

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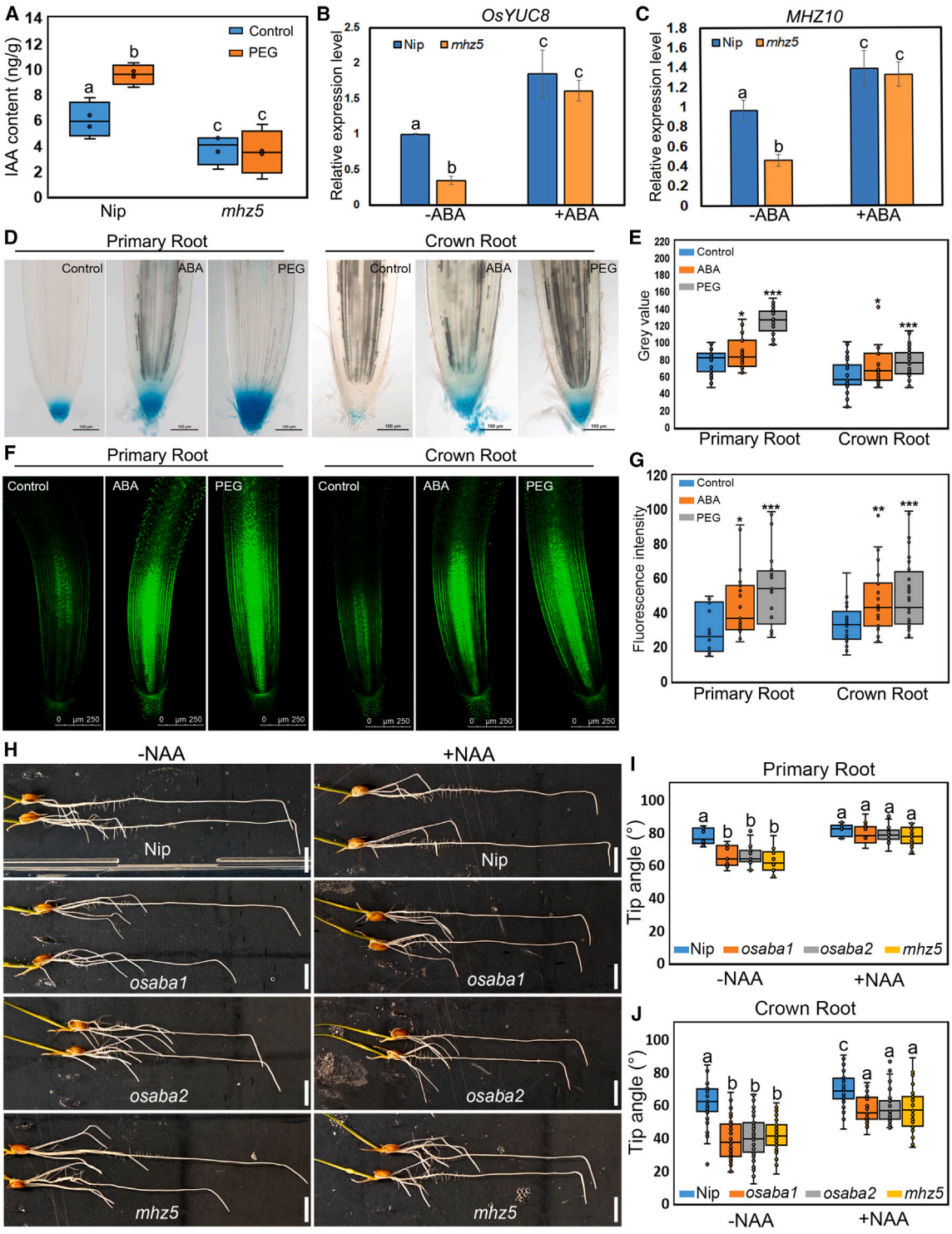

Figure 4. ABA modulates auxin biosynthesis to control root angle under drought stress

(A) Measured auxin levels in primary root tips of WT and *mhz5* mutant seedlings under control and 10% PEG treatment. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n = 4$, n represents the total number of biological replicates).

(B) Bar graph showing relative expression (fold-change [FC]) of auxin biosynthetic genes (*OsYUC8*) in root tips (1 cm) of Nip and *mhz5* primary roots, with or without 100 nM ABA treatment. The experiment was performed for three biological replicates, each comprising approximately 10 root tips. Data are presented as means ± SE. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n = 3$, n represents the total number of biological replicates).

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DNA [T-DNA] insertion mutant lacks any transcripts) shows defective root gravitropic response[21] (Figure S8). Given the close phylogenetic relationship between OsbZIP46 and OsbZIP23, we further constructed the double mutant of *osbzip23 osbzip46* using CRISPR-Cas9 technology, but it did not show defective root gravitropism either. Approximately 89 bZIP transcription factors exist in rice, and many of them are involved in ABA-mediated signaling.[44] This suggests that other bZIP transcription factors or other novel linkages between ABA and auxin biosynthesis may contribute to the regulation of root angle. Moreover, given that *MHZ10* expression is downregulated in the ABA biosynthetic mutant *mhz5* and upregulated by ABA treatment (Figures 4C, 4F, and 4G), MHZ10 may be a downstream target of ABA signaling. *mhz10-1* showed significantly reduced root gravitropism and exogenous ABA application could not restore this defect (Figures S7D–S7K), suggesting that MHZ10 acts downstream of ABA in root gravitropism. There are 3 homologs (OsTAR1, OsTARL1, and OsTARL2) of MHZ10/OsTAR2 in rice, with distinct roles in tiller and grain development.[55,56] Thus, it is possible that *MHZ10* may be induced by a novel transcription factor such as OsbZIPs in the ABA signaling pathway, regulating root angle under drought stress.

The root system consists of different root types, including primary, seminal, lateral, and crown roots, each exhibiting different growth angles. Primary roots typically grow vertically, while other root types display non-vertical growth. However, the molecular mechanisms underlying this phenotype remain poorly understood. Some studies have uncovered the involvement of auxin distribution in maintaining non-vertical growth in lateral roots in *Arabidopsis*.[57] In this study, we found that ABA-mediated auxin biosynthesis plays a positive role in root gravitropism, influencing crown root angle. Crown roots with a reduced auxin response (Figures 5G, 5H, 5Q, and 5R) in the epidermis exhibit a non-vertical growth angle. Moreover, *mhz5* crown roots, which show a weaker auxin response, show a shallower growth angle than WT crown roots (Figures 2 and 5). These findings suggest that reduced auxin response may be a key factor contributing to the impaired gravitropic response observed in crown roots of rice.

Collectively, our work uncovered a previously unrecognized function of ABA in regulating root angle by inducing auxin biosynthesis under drought stress, a mechanism that appears to be conserved in rice and maize. This model has the potential to improve crop yield by promoting the development of steep RSA in drought-prone areas.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Guoqiang Huang (huang19880901@sjtu.edu.cn).

Materials availability

DNA constructs and transgenic rice seeds generated in this study are available from the lead contact, Guoqiang Huang, upon request.

Data and code availability

- All data are available in the main text or the supplemental information.
- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, G.H., R.B., X.K., and M.B.; investigation, Y.X., X.S., P.M., S.Y., and Q.L.; formal analysis, Y.X., X.S., P.M., D.T., and G.H.; resources, G.H., M.B., and R.B.; writing—original draft, Y.X. and G.H.; writing—review

(C) Bar graph showing relative expression (fold-change [FC]) of auxin biosynthetic genes (*MHZ10*) in root tips (1 cm) of Nip and *mhz5* primary roots, with or without 100 nM ABA treatment. The experiment was performed for three biological replicates, each comprising approximately 10 root tips. Data are presented as means ± SE. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n = 3$, n represents the total number of biological replicates).
(D) Representative GUS staining images of primary and crown roots of 4-day-old seedlings of *proYUC8-GUS* transgenic plants, with or without 10 nM NAA or 10% PEG treatment for 6 h. Scale bars, 100 μm.
(E) Boxplot showing the quantitative intensity of GUS staining in primary and crown roots of 4-day-old seedlings of *proYUC8-GUS* transgenic plants, with or without 10 nM NAA or 10% PEG treatment for 6 h. Data are presented as means ± SE. * and *** indicate significant differences (Student's t test: $^{*}p < 0.05$, $^{***}p < 0.001$, $n > 15$, n represents the total number of roots).
(F) Representative confocal images of primary and crown roots of 4-day-old seedlings of *proMHZ10-VENUS-N7* transgenic plants, with or without 100 nM ABA or 10% PEG treatment for 6 h. Scale bars, 250 μm.
(G) Boxplot showing the quantitative intensity of fluorescence signals in primary and crown roots of 4-day-old seedlings of *proMHZ10-VENUS-N7* transgenic plants, with or without 10 nM NAA or 10% PEG treatment for 6 h. Data are presented as means ± SE. *·** and *** indicate significant differences (Student's t test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $n > 15$, n represents the total number of roots).
(H) Representative images of root phenotypes of 4-day-old seedlings of Nip, *osaba1*, *osaba2*, and *mhz5*, with or without 10 nM NAA treatment after 12 h gravistimulation. Scale bars, 1 cm.
(I) Tip angle analysis of Nip, *osaba1*, *osaba2*, and *mhz5* primary root under control and 10 nM NAA treatment with 12 h gravistimulation. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of primary roots).
(J) Tip angle analysis of Nip, *osaba1*, *osaba2*, and *mhz5* crown root under control and 10 nM NAA treatment with 12 h gravistimulation. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of crown roots).
See also Figures S6–S9.

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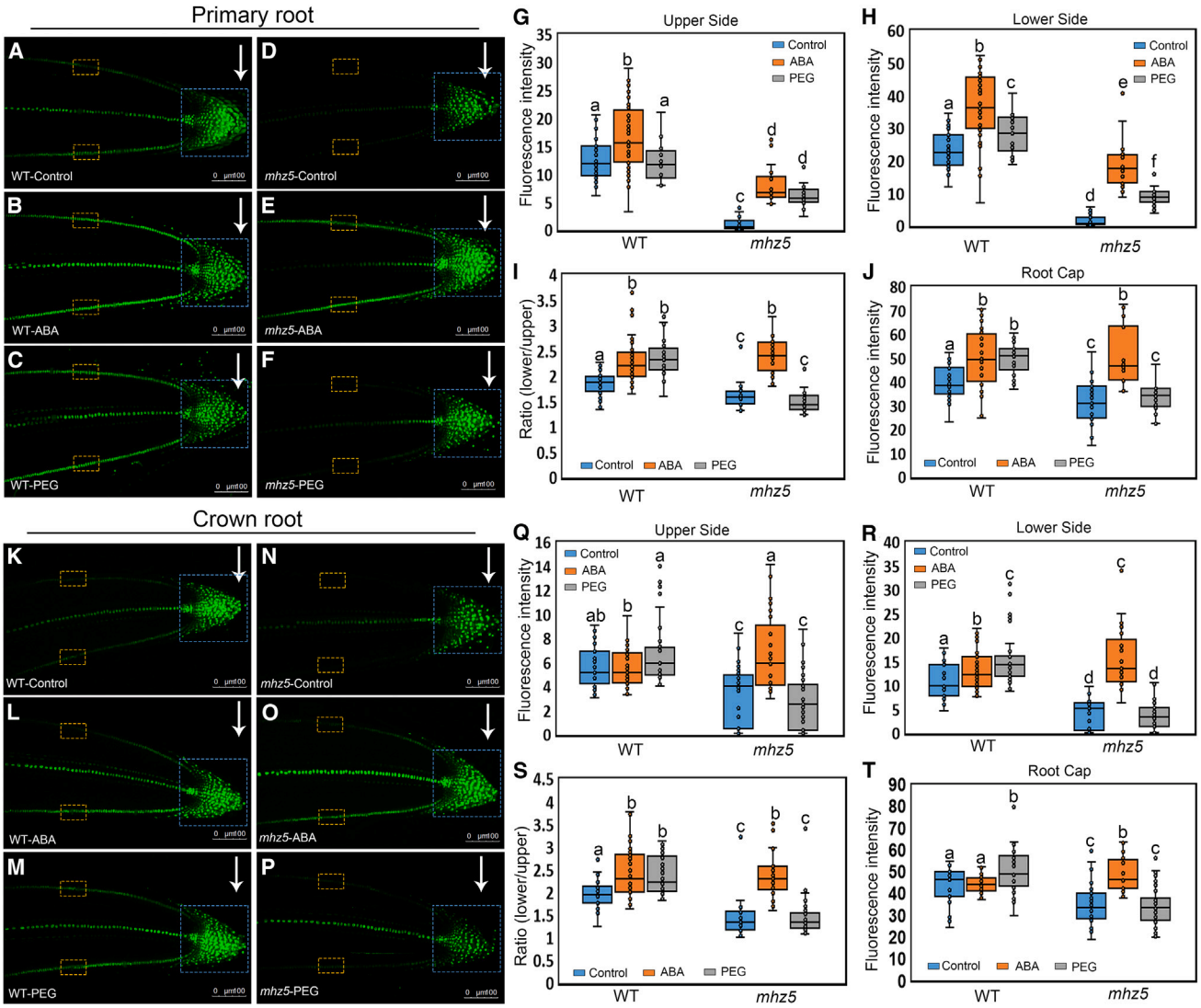

Figure 5. Auxin response was reduced in *mhz5*

(A–F) Representative confocal images of DR5-VENUS in 4-day-old seedlings of WT (A–C) and *mhz5* (D–F) primary roots after 4 h gravistimulation, with or without 10% PEG or 100 nM ABA treatment. White arrows mean the directions of gravity. Orange and blue dotted boxes show the region of interests (ROI). Scale bars, 100 μm.

(G) Boxplot showing the quantitative intensity of fluorescence signals in the upper (left) ROI of primary roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(H) Boxplot showing the quantitative intensity of fluorescence signals in the lower (right) ROI of primary roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(I) Boxplot showing the ratio of lower/upper of DR5 fluorescence signals in ROI of primary roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(J) Boxplot showing the quantitative intensity of fluorescence signals in the root cap (blue dotted boxes) of primary roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(K–P) Representative confocal images of DR5-VENUS in 4-day-old seedlings of WT (K–M) and *mhz5* (N–P) crown roots after 4 h gravistimulation, with or without 10% PEG or 100 nM ABA treatment. White arrows mean the directions of gravity. Orange and blue dotted boxes show the region of interests (ROI). Bars, 100 μm.

(Q) Boxplot showing the quantitative intensity of fluorescence signals in the upper (left) ROI of crown roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(R) Boxplot showing the quantitative intensity of fluorescence signals in the lower (right) ROI of crown roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(S) Boxplot showing the ratio of lower/upper of DR5 fluorescence signals in ROI of crown roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

(T) Boxplot showing the quantitative intensity of fluorescence signals in the root cap (blue dotted boxes) of crown roots. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 10$, n represents the total number of roots).

See also Figures S6–S9.

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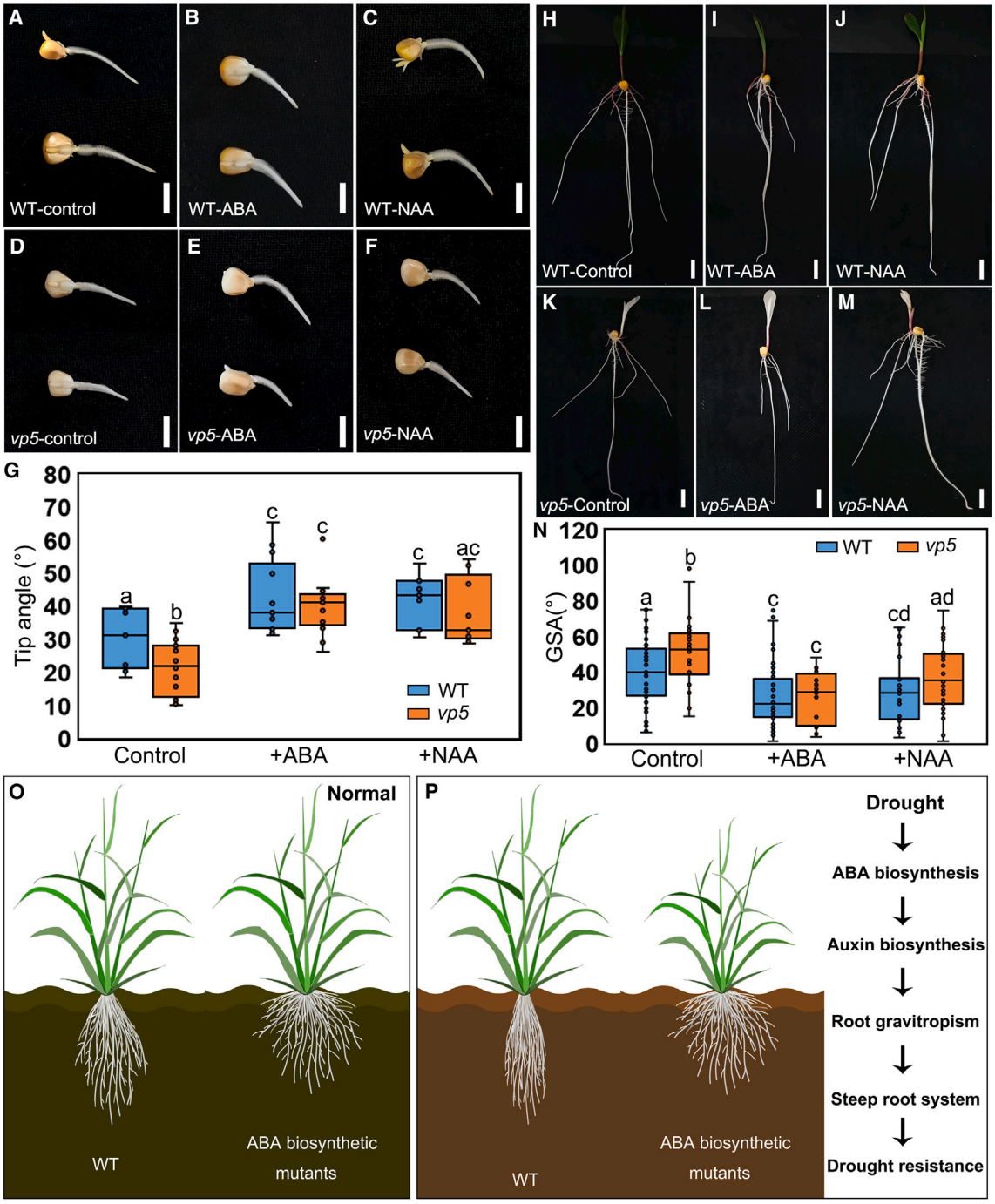

Figure 6. The function of ABA in root gravitropism is conserved in maize

(A–F) Representative images of roots of 1-day-old seedlings of WT after 12 h gravitropic responses while growing in control (A) or with 1 μM ABA (B) or 100 nM NAA (C) treatment, and *vp5* in control (D) or with 1 μM ABA (E) or 100 nM NAA (F) treatment. Scale bars, 1 cm.

(G) Tip angle analysis of WT and *vp5* primary root after 12 h gravistimulation, with or without 1 μM ABA and 100 nM NAA treatment. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of primary roots).

(H–M) Representative images of root systems of WT and *vp5*, grown with or without 1 μM ABA and 100 nM NAA treatment for 6 days. Scale bars, 2 cm.

(N) GSA analysis of WT and *vp5* grown for 6 days, with or without 1 μM ABA and 100 nM NAA treatment. Different letters indicate significant differences (one-way ANOVA: $p < 0.05$, $n > 15$, n represents the total number of seminal roots).

(O and P) Model of ABA regulating root angle by inducing auxin biosynthesis under drought stress. ABA biosynthetic mutants show shallower root systems compared with WT under normal conditions (O). However, drought stress induces ABA biosynthesis, which increases local auxin levels, enhances root gravitropism, and promotes the formation of steep root systems (P).

See also Figures S6–S9.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
E. coli (strain DH5α)	Sangon biotech	Cat#B528413
Agrobacterium tumefaciens (strain EHA105)	Weidi bio	Cat#AC1010
Critical commercial assays		
KOD One™ PCR Master Mix	TOYOBO	Cat#KMM-101
ClonExpress II One Step Cloning Kit	Vazyme	Cat#C112-02
TRNzol Universal RNA Reagent	TIANGEN	Cat#DP424
ToloScript ALL-in-one RT EasyMix for qPCR	TOLOBIO	Cat#22107
2 X Q5 SYBR qPCR Master Mix (Universal)	TOLOBIO	Cat#22208
Deposited data		
Analyzed Data	This paper	N/A
Experimental models: Organisms/strains		
Rice: Nipponbare (wild type)	Widely used	N/A
Rice: DongJin (wild type)	Widely used	N/A
Rice: Hwayoung (wild type)	Widely used	N/A
Rice: *mhz4*/Nip	Ma et al.[36]	N/A
Rice: *mhz5*/Nip	Yin et al.[37]	N/A
Rice: *osaba1*/Nip	Agrawal et al.[38]	N/A
Rice: *osaba2*/Nip	Liao et al.[39]	N/A
Rice: *osyuc8-2*/HWY	Qin et al.[58]	N/A
Rice: *osaux1-3*/DJ	Giri et al.[59]	N/A
Rice: *mhz10-1*/Nip	Zhou et al.[55]	N/A
Rice: *osabi3*/ZH11	Li et al.[45]	N/A
Rice: *osabi5c1*/ZH11	Li et al.[45]	N/A
Rice: *osabi5c2*/ZH11	Li et al.[45]	N/A
Rice: *osbzip23*/ZH11	Xiang et al.[60]	N/A
Rice: *osbzip46-1*/ZH11	Qin et al.[43]	N/A
Rice: *osbzip46-2*/ZH11	Qin et al.[43]	N/A
Rice: *osbzip23 osbzip46-1*/ZH11	This paper	N/A
Rice: *osbzip23 osbzip46-2*/ZH11	This paper	N/A
Rice: *osbzip23 osbzip46-3*/ZH11	This paper	N/A
Rice: DR5-VENUS	Huang et al.[61]	N/A
Rice: DR5-VENUS/*mhz5*	This paper	N/A
Rice: *ProOsYUC8-GUS*	This paper	N/A
Rice: *ProMHZ10-VENUS-N7*	This paper	N/A
Maize: *vp5*	Wilson et al.[62]	N/A
Oligonucleotides		
Ubiquitin RT-qPCR Forward: GAGCCTCTGTTCGTCAAGTA	This paper	N/A
Ubiquitin RT-qPCR Reverse: ACTCGATGGTCCATTAAACC	This paper	N/A
MHZ10 RT-qPCR Forward: GATGACCACTCCTCTTCGCC	This paper	N/A
MHZ10 RT-qPCR Reverse: TCCACATCCTCCCTATCGCA	This paper	N/A
OsYUC8 RT-qPCR Forward: TGGTCTCAAGAGGCCCAAAC	This paper	N/A
OsYUC8 RT-qPCR Reverse: ACATTGCTTCTGTAGCCGGT	This paper	N/A
OsbZIP23 CRISPR Forward: TA GGTCTCCCTGCC TCTGAACGTTTTAGAGCTAGAA	This paper	N/A

(*Continued on next page*)

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
OsbZIP23 CRISPR Reverse: CG GGTCTCA GCA GGGGTCGCT TGCACCAGCCGGG	This paper	N/A
OsbZIP46 CRISPR Forward: TA GGTCTCC GAGAA GGATTTC GTTTTAGAGCTAGAA	This paper	N/A
OsbZIP46 CRISPR Reverse: CG GGTCTCA TCTCGGCGCTTC TGCACCAGCCGGG	This paper	N/A
OsbZIP23 CRISPR mutants identification Forward: ATGGATTTTCCGGGAGGGA	This paper	N/A
OsbZIP23 CRISPR mutants identification Reverse: TCTCTCGACAACCTTCTCGAT	This paper	N/A
OsbZIP46 CRISPR mutants identification Forward: ATCTAATCCAATCGGCCGA	This paper	N/A
OsbZIP46 CRISPR mutants identification Reverse: TGCAGTGGCTATTAGGCAT	This paper	N/A
mhz5 mutant identification Forward: CCCTACAGTGCTTGACCCAT	This paper	N/A
mhz5 mutant identification Reverse: AGGAAAACAGCTGTCGCCAA	This paper	N/A
osabi3 mutant identification Forward: TTCGCTGACGATACCTTCCC	This paper	N/A
osabi3 mutant identification Reverse: GTACAGTCGTCCGCCTGATG	This paper	N/A
osabi5 mutant identification Forward: TGGCATCGGAGATGAGCAAG	This paper	N/A
osabi5 mutant identification Reverse: GCGGCAAGGAAAATGATCCC	This paper	N/A
Software and algorithms		
ImageJ	Widely used	https://imagej.nih.gov/ij/
Excel 2019	Widely used	N/A
SPSS, version 26.0 (SPSS Inc., Chicago, IL, USA)	Widely used	N/A

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant materials and growth conditions

The rice (*Oryza sativa* L.) mutants *osaba1*,[38] *osaba2*,[39] *mhz4*,[36] *mhz5*,[37] *osaux1-3*,[59] *ostar2/mhz10-1*,[55] *osyuc8-2*,[58] *osabi3*,[45] *osabi5c1*,[45] *osabi5c2*,[45] *osbzip23*,[60] *osbzip46-1*,[43] *osbzip46-2*[43] and the maize (*Zea mays*) ABA defective mutant *vp5*[62] have been described in previous studies. The backgrounds of *osaba1*, *osaba2*, *mhz4*, *mhz5, osbzip46-1, osbzip46-2* and *ostar2/mhz10-1* are Nipponbare (Nip) (*Oryza sativa*, *Japonica*), and *osabi3, osabi5c1, osabi5c2, osbzip23* are Zhonghua11 (ZH11) (*Oryza sativa*, *Japonica*), and *osaux1-3* is Dongjin (DJ) (*Oryza sativa*, *Japonica*). *osbzip23 osbzip46-1*, *osbzip23 osbzip46-2* and *osbzip23 osbzip46-3* were generated in ZH11 by CRISPR/Cas9 technology. The genetic transformation of rice was performed by EDGENE BIOT Company (http://www.edgene.com.cn/nav/1.html). Maize *vp5* mutant was maintained as a heterozygote and homozygous *vp5* was identified by white kernels and white leaves.[48] For seeds propagation, rice and maize plants were cultivated in Shanghai (30°N, 121°E) and Sanya (18°N, 109°E), China, in summer and winter, respectively. Seeds were germinated on moist paper for 4 d in a growth chamber (12 h photoperiod at 300 μmol $m^{-2}s^{-1}$ light intensity, 70% relative humidity).

METHOD DETAILS

Root system observation

To observe root system of mature plants in the soil, we used a modified earth cutting method as previously described.[21] Seedlings of rice were grown in the paddy field in Shanghai in summer for one month. For drought treatment, plants were not irrigated for the following one month and rewatered. And the control group was kept with good irrigation. One month later, all plants with soil monoliths (20 cm × 20 cm × 5 cm) were picked up from the field and fixed on plates with nails. Then, we used running water to carefully wash the soil from the plates. GSA was calculated at the emergence site of crown or seminal roots.

To observe the root system of seedlings in the soil, we used the technology of X-ray CT imaging as previously described.[61] Equally germinated seedlings were transplanted to 3D-printed columns (33 mm diameter × 100 mm height) filled with soil. The plants were cultivated 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. Seven-day-old seedlings were scanned using a Phoenix v|tome|x M 240 kV X-ray μCT system (Waygate Technologies [a Baker Hughes business]) at the Hounsfield Facility (University of Nottingham, Sutton Bonington 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 kV and 200 μA, respectively. The detector exposure time was 131 msec, and the voxel resolution was 57 μm. Scan time was 5 min. Each root was segmented into sections of 5 mm and GSA was calculated for each section.

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We used seed germination bags to observe root systems of seedlings in laboratory.[21] Equally germinated seeds were transferred to the seed germination bag (length: 30 cm, width: 25 cm) containing filter paper with 10 millilitres (mL) of tap water in darkness at 28°C for 7 days in rice and 5 days in maize. For drought treatment, we replaced 10 mL water with 8 mL water, the roots were positioned directly on moist paper. And for PEG, NAA and ABA treatment, we replaced tap water with 10% PEG, 10 nM NAA and 100 nM ABA, respectively. The GSA was measured at the emergence site of crown or seminal roots.

Hormone measurements

Seeds of wild type (cv. Nipponbare) and *mhz5* were germinated on moist paper for 4 days in darkness and then transplanted to 96-well boxes containing 1 liter (L) tap water. After 3 days of growth, seedlings were treated with or without 10% PEG 6000 for another 3 days, respectively. All primary root tips (2 cm) were cut with razor blades quickly and stored in liquid nitrogen. The levels of auxin and ABA were measured and analyzed by Metware Company (https://www.metware.cn/).

RNA Isolation and RT-qPCR

Equally germinated seeds of Nip and *mhz5* were transferred to 96-well plates with 1 liter tap water for 5 days and 1 cm tips of primary roots were harvested rapidly. Total RNA was extracted by TRNzol Universal RNA Reagent (TIANGEN, #DP424) according to the standard protocol. For quantitative RT-PCR, cDNA was synthesized by RNA using ToloScript ALL-in-one RT EasyMix for qPCR (TOLOBIO, #22107). RT–qPCR assays were performed with 2 X Q5 SYBR qPCR Master Mix (Universal) (TOLOBIO, #22208) on a real-time PCR system (Roche, LightCycler® 96 Instrument) according to the specification. The expression levels of target genes were normalized to rice OsUBIQUITIN (OsUBQ, Os05g06770). All RT-qPCR experiments were performed with at least three biological replicates and three technical replicates. RT-qPCR primers are listed in Key Resources Table.

Gravitropism analysis and hormone treatment

Equally germinated seeds of rice were hydroponically cultivated with 18 h light and 6 h dark at 28 °C for 4 days and then were transplanted on 1% agar with or without 10 nM NAA or 100 nM ABA for 1 h vertically in the dark. Then the plates were widdershins rotated to place them horizontally. Photographs were taken per hour. The angle between the growing direction of the root tip and the horizontal line was measured by ImageJ (http://rsb.info.nih.gov/ij/).

For maize, equally germinated seeds were transplanted on 1% agar with or without 100 nM NAA or 1 μM ABA for 1 day vertically placed in the dark. Then, the plates were widdershins rotated for horizontally place of roots. Photographs were taken per hour. The angle between the growing direction of the root tip and the horizontal line was measured by ImageJ (http://rsb.info.nih.gov/ij/).

Confocal imaging

The DR5-VENUS reporter was crossed with *mhz5* to generate DR5 reporter lines in *mhz5* mutant and wild type. Equally germinated seeds were transferred into 96-well plates for 4 days. Then seedlings were vertically placed on 1% agar with or without 10 nM NAA or 100 nM ABA for 1 h. For PEG treatment, seedlings were treated with 10% PEG the day before gravitropic treatment. Then the plates were rotated anticlockwise. After 4 h gravitropic growth, root tips (the bending site was included to keep track of the direction of gravity) were harvested and fixed by 4% paraformaldehyde. After thrice washing by PBS solution and 3 days clear by ClearSee (xylitol powder [10% (w/v)], sodium deoxycholate [15% (w/v)] and urea [25% (w/v)] in water),[63] the fluorescence signal in roots was observed and analyzed via Leica Laser Scan Microscope (SP5) using an excitation wavelength of 514 nm, intensity less than 10, collection wavelength of 520 to 550 nm, and gain less than 800.

For *proMHZ10-VENUS-N7* reporter, equally germinated seeds were transferred into 96-well plates for 4 days, and then treated with 100 nM ABA or 10% PEG for 6 h. Root tips were harvested and fixed by 4% paraformaldehyde. After thrice washing with PBS solution and 3 days clear by ClearSee (xylitol powder [10% (w/v)], sodium deoxycholate [15% (w/v)] and urea [25% (w/v)] in water),[63] the fluorescence signal in roots was observed and analyzed via Leica Laser Scan Microscope (SP5) using an excitation wavelength of 514 nm, intensity less than 10, collection wavelength of 520 to 550 nm, and gain less than 800.

Histochemical staining of GUS

Equally germinated seeds of *proYUC8-GUS* transgenic plants were transferred into 96-well plates for 4 days, and then treated with 100 nM ABA or 10% PEG for 6 h. Root tips were harvested and incubated in sodium phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 and 2 mM X-Gluc at 37°C for 12 h. After the samples were rinsed with 70% ethanol until the tissue cleared, they were photographed. The images of rice root autofluorescence were taken under a microscope (Nikon ECLIPSE Ni). The grey value of GUS staining was measured by ImageJ (http://rsb.info.nih.gov/ij/).

Accession numbers

Sequence data can be found in the GenBank/EMBL data libraries: *MHZ4* (Os01g0128300); *MHZ5* (Os11g0572700); *OsABA1* (Os04g0448900); *OsABA2* (Os03g0810800); *OsYUC8* (Os03g0162000); *MHZ10/OsTAR2* (Os01g0169800); *OsAUX1* (Os01g0856500); *OsbZIP23* (Os02g0766700); *OsbZIP46* (Os06g0211200); *OsABI3* (Os01g0911700); *OsABI5* (Os01g0859300); *VP5* (Zm00001eb006300).

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QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed using *Excel* (2023) and the statistical software program named SPSS, version 26.0 (SPSS Inc., Chicago, IL, USA). Data collection and analyses were performed by investigators blinded to the experimental conditions. Experiments were randomized whenever possible. All experiments were replicated in multiple subject rice plants with similar results. All of the statistical details, including the statistical tests used, exact value of n, etc., can be found in main (or supplementary) figure legends. $p < 0.05$ was regarded as statistically significant.