Plant Biotechnology Journal

Plant Biotechnology Journal (2015) **13**, pp. 105–116 doi: 10.1111/pbi.12241

A late embryogenesis abundant protein HVA1 regulated by an inducible promoter enhances root growth and abiotic stress tolerance in rice without yield penalty

Yi-Shih Chen[1,2], Shuen-Fang Lo[1,3], Peng-Kai Sun[1], Chung-An Lu[2], Tuan-Hua D. Ho[3,4,5,*] and Su-May Yu[1,3,5,*]

[1]*Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan*
[2]*Department of Life Sciences, National Central University, Jhongli City, Taiwan*
[3]*Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan*
[4]*Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan*
[5]*Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan*

Received 7 May 2014;
revised 6 July 2014;
accepted 7 July 2014.
**Correspondence* (Tel 886-2-2788-2695;
fax 886-2-2782-6085;
email sumay@imb.sinica.edu.tw) and
(Tel 886-2-2787-1090;
fax 886-2-2782-1605;
email tho@gate.sinica.edu.tw;
ho@biology2.wustl.edu)

Accession number: HVA1 (CAA31853); CRL1 (AB200234); CYCB1.1 (AK111939); CYCB2.1 (AK070211); CYCB2.2 (AK070518); MSU_Gene_ID: PIN1b (LOC_Os02g50960); PIN2 (LOC_Os06g44970); PIN9 (LOC_Os01g58860).

Keywords: rice, barley HVA1, stress-inducible composite promoter, ABA, lateral and primary root growth, abiotic stress tolerance.

Summary

Regulation of root architecture is essential for maintaining plant growth under adverse environment. A synthetic abscisic acid (ABA)/stress-inducible promoter was designed to control the expression of a late embryogenesis abundant protein (HVA1) in transgenic rice. The background of HVA1 is low but highly inducible by ABA, salt, dehydration and cold. HVA1 was highly accumulated in root apical meristem (RAM) and lateral root primordia (LRP) after ABA/stress treatments, leading to enhanced root system expansion. Water-use efficiency (WUE) and biomass also increased in transgenic rice, likely due to the maintenance of normal cell functions and metabolic activities conferred by HVA1 which is capable of stabilizing proteins, under osmotic stress. HVA1 promotes lateral root (LR) initiation, elongation and emergence and primary root (PR) elongation via an auxin-dependent process, particularly by intensifying asymmetrical accumulation of auxin in LRP founder cells and RAM, even under ABA/stress-suppressive conditions. We demonstrate a successful application of an inducible promoter in regulating the spatial and temporal expression of HVA1 for improving root architecture and multiple stress tolerance without yield penalty.

Introduction

Plant root architecture is essential for water and nutrient uptake, anchorage and interactions with microbes in the soil, functions that impact growth rate, yield and abiotic stress tolerance. Root branching is enhanced by both water and nutrient deficiencies, and such root developmental plasticity offers one of the major acclimation strategies for plants to adapt to limited supplies of water and nutrients in the soil (Lopez-Bucio *et al.*, 2003; Potters *et al.*, 2007). Understanding the mechanism regulating root architecture is of great agronomic importance; for example, the majority of drought-resistant rice varieties have a deeper and more highly branched root system than drought-sensitive varieties (Price *et al.*, 1997; Uga *et al.*, 2013). However, detailed mechanisms underlying how environmental cues regulate root architecture remain mostly unclear.

Studies in Arabidopsis have significantly advanced our knowledge of mechanisms controlling root development (Lavenus *et al.*, 2013; Malamy, 2005; Peret *et al.*, 2009; Potters *et al.*, 2007); however, similar studies in cereals are relatively limited (Coudert *et al.*, 2010). Unlike Arabidopsis, in which the PR iteratively branches to generate several orders of lateral roots, the cereals have several types of branched roots including shoot-born crown roots and root-born lateral roots. Although several homologous genes that play similar roles regulating root formation between Arabidopsis and cereals have been identified, distinct hormonal and developmental pathways have also been identified in cereals (Orman-Ligeza *et al.*, 2013). Auxin acts as a common integrator for many endogenous and environmental signals regulating LR development in both dicots and monocots (Lavenus *et al.*, 2013; Orman-Ligeza *et al.*, 2013). In Arabidopsis, ABA stimulates main root elongation in response to drought and osmotic stresses, yet ABA and auxin signals seem to act antagonistically during LR initiation, with ABA acting as a repressor while auxin as a promoting agent (De Smet *et al.*, 2006). However, low ABA concentrations have also been shown to be promotive in root development in rice and Arabidopsis (Xu *et al.*, 2013). Both auxin and ABA play essential roles regulating root development, but how they interplay to regulate root development in response to abiotic stress is unclear neither.

The current global climate changes tend to shift weather to more extreme variations, which aggravate the world crop productivity that has already plateaued (Lucas, 2003). Rice is the major dietary staple for nearly half of humanity. However, of

the 130 million hectares of rice lands, close to 30% is affected by drought (Tuong and Bouman, 2003), 20% by salinity (Negrao *et al.*, 2011) and 10% by low temperature (Sthapit and Witcombe, 1998). The development of new strategies for breeding rice with tolerance to multiple stresses that still maintain high productivity remains an important subject of research. Over-accumulation of osmoprotectants or proteins has been widely pursued as effective strategies in improving plant stress tolerances (Ahuja *et al.*, 2010; Bartels, 2001; Ho and Wu, 2004; Yamaguchi-Shinozaki and Shinozaki, 2006). However, under normal environmental conditions, constitutive overproduction of these compounds or proteins consumes extra energy and often results in plant growth retardation and yield penalty (Dubouzet *et al.*, 2003; Hsieh *et al.*, 2002; Kasuga *et al.*, 1999). To minimize such negative effect while still improving stress tolerance of plants, there is promise in inducible promoters that control the accumulation of an osmoprotectant or a protein, ensuring that stress tolerance mechanisms are initiated only under stress conditions (Garg *et al.*, 2002; Kasuga *et al.*, 1999; Lee *et al.*, 2003; Morran *et al.*, 2011).

Late embryogenesis abundant (LEA) proteins are highly accumulated in seeds at the onset of desiccation and in plant vegetative tissues in response to water deficit (Dure, 1992). HVA1 is a group 3 small LEA protein specifically expressed in barley seeds during late stages of seed development undergoing desiccation and is rapidly induced in young barley seedlings, especially in roots, by abiotic stresses (Hong *et al.*, 1992). Expression of the rice *HVA1* homologue, *LEA3*, is also highly induced in roots of rice seedlings in response to ABA and salt (Moons *et al.*, 1997). HVA1 contains an 11-amino acid consensus motif that is repeated nine times, potentially forming an amphipathic α-helical dimer suitable for accommodating positively and negatively charged ions, and has therefore been proposed to function in sequestering ions (Dure, 1993). Most LEA proteins belong to a class of proteins called hydrophilins that are proposed to exist as unstructured proteins in aqueous solutions and thus function in protecting cells from damages caused by water limitation (Olvera-Carrillo *et al.*, 2011). Many *in vitro* experiments have demonstrated that LEA could prevent the inactivation of enzymes and aggregation of proteins or maintain the functional membrane structure under dehydration and freeze–thaw conditions (Battaglia and Covarrubias, 2013). Overexpression of a soybean LEA3 confers high salt and extreme temperature tolerance in *E. coli* (Liu *et al.*, 2010). A shrimp LEA3-like protein confers enhanced viability in *Drosophila melanogaster* cells after desiccation and hyperosmotic stress (Marunde *et al.*, 2013). Ectopic expression of HVA1 also protects plants against drought, salt and osmotic stresses in various monocot and dicot plant species (Baena-Gonzalez *et al.*, 2007; Battaglia and Covarrubias, 2013; Nguyen and Sticklen, 2013; Xu *et al.*, 1996); however, the mechanism related to HVA1 conferring stress tolerance in transgenic plants is unknown.

Many ABA-inducible promoters contain a conserved ABA response element (ABRE) with an ACGT core and a coupling element (CE) forming an ABA response complex (ABRC) (Shen *et al.*, 1993). Single and multiple copies of ABRC1 from the *HVA22* promoter have been used to manipulate stress-inducible gene expression and stress tolerance in transgenic rice and tomato (Garg *et al.*, 2002; Lee *et al.*, 2003), but high background of transgene expression is present under nonstress conditions (Garg *et al.*, 2002).

In this study, an ABRC-based synthetic composite promoter *3xABRC321* was designed with minimal background activity but capable of conferring spatial and temporal activation of transgene expression under stress conditions. Ectopic expression of *3xABRC321:HVA1* led to a significant increase in the expansion of primary and branch root systems in response to ABA/stress and thus enhanced tolerance to drought, salt and cold stresses and WUE in transgenic rice. HVA1 was found to promote auxin accumulation in meristematic tissues that initiate LR and PR growth through an auxin-dependent process despite the presence of ABA, indicating that the ABA-mediated environmental signal crosstalks with the auxin-mediated developmental signal to regulate root architecture.

Results

The synthetic promoter *ABRC321* has low background activity but is highly inducible by ABA and abiotic stresses in transgenic rice

ABRC321 was generated by fusion of CE and ABRE from *HVA1* and *HVA22* promoters (Shen *et al.*, 1996) (Figure 1a). The reporter *GUS* was fused downstream of 1–3 tandem repeats of *ABRC321* promoter (Figure S1a) and used for rice stable transformation. The GUS activity in leaves and roots controlled by *3xABRC321* had relatively low background but was highly inducible by ABA (Figure S1c).

The *3xABRC321* promoter was then used to control the expression of HVA1 (Figure S1b) in transgenic rice. The expression of *3xABRC321:HVA1* was induced in leaves and roots of transgenic seedlings, with levels generally higher in roots, and the expression of endogenous LEA3 was also induced in roots of wild-type (WT) rice (Figure 1b). The accumulation of HVA1 in seedlings was induced and increased with time up to 20 h under ABA, NaCl, cold and dehydration treatments (Figure 1c), but decreased gradually after relief from these stresses (Figure 1d). The half-life of HVA1 was estimated to be 78.7 and 157.8 h, in contrast to that of tubulin to be 60.1 and 46.3 h, in the absence and presence of ABA, respectively (Figure 1e). These studies indicate that both enhanced mRNA transcription and protein stability of HVA1 may account for the high-level accumulation of HVA1 in response to ABA and abiotic stresses.

3xABRC321:HVA1 enhances root growth in transgenic rice under low ABA concentrations and osmotic stress

Rice contains seminal, crown and LRs (Figure S2). Crown roots of transgenic rice were longer, and LR numbers were greater in transgenic lines than in WT even without ABA and were further increased by 0.1 μM ABA in transgenic lines (Figure 2a,b). Although LRs became shorter and thicker and LR density decreased with ABA concentrations exceeding 0.5 μM, all roots in transgenic plants were still longer and LR number and density greater than WT at all ABA concentrations (Figure 2a,b).

Transgenic seedlings were also treated with 400 mM sorbitol, which mimics osmotic stress. LR number and density of crown and seminal root in transgenic lines were increased with sorbitol (Figure 3a). All roots in transgenic lines were longer and had higher LR density than those of WT with sorbitol and in transgenic lines were all longer and LR density higher with sorbitol (Figure 3b). Transgenic lines also have higher dry and fresh weights in shoots and roots than WT with sorbitol (Figure 3c).

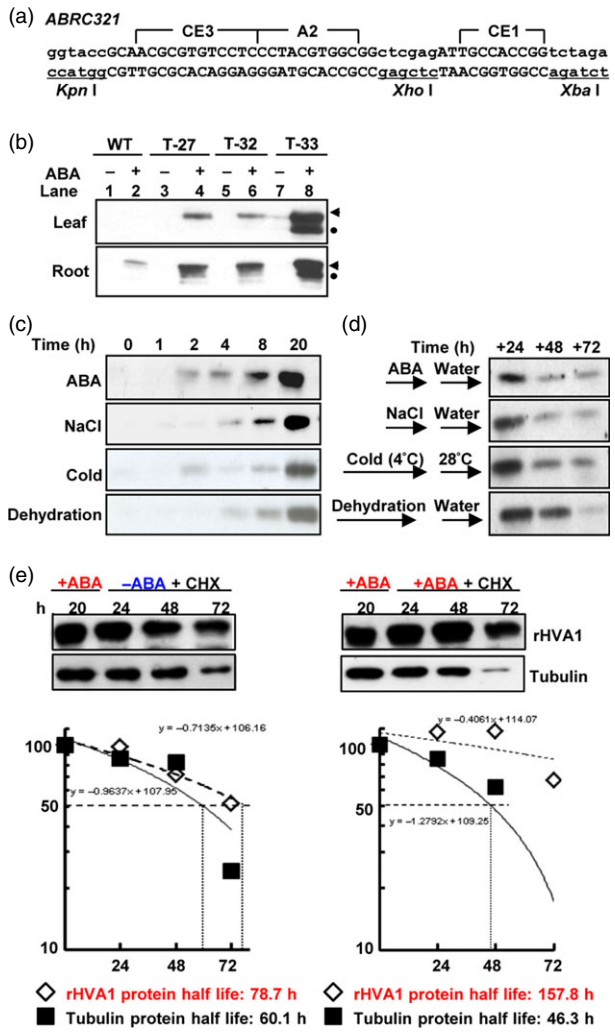

Figure 1 *3xABRC321-HVA1* is highly inducible by abiotic stress, and the half-life of HVA1 is enhanced by ABA in transgenic rice. Ten-day-old seedlings of Tainung 67 transgenic lines were treated with various abiotic stresses. Total proteins were extracted from leaves and subjected to protein gel blot analysis using the antibarley HVA1 and anti-α-tubulin antibodies. (a) Nucleotide sequence of *ABRC321*. (b) Seedlings were treated with or without 10 μM ABA for 24 h. Triangle indicates the 27-kD HVA1, and dot indicates a smaller protein of 22 kD which was likely the degradation form of HVA1. (c) Seedlings were treated with 10 μM ABA, 200 mM NaCl or dehydration (slow air dry) at 28 °C or with cold (4 °C) for various lengths of time. (d) Seedlings were treated with various stresses for 20 h and then transferred to water at 28 °C for indicated time. Five seedlings were used for each treatment at each time point in (b)–(d). (e) Seedlings were incubated with 10 μM ABA for 20 h to allow the accumulation of HVA1, and then, seedlings were transferred to freshwater containing 200 μM cycloheximide (in 100% EtOH) with or without 10 μM ABA for up to 72 h. Levels of HVA1 shown in the protein gel blot were quantified densitometrically. The relative HVA1 and α-tubulin levels were then determined by a linear regression analysis, and the graph was plotted using a linear regression algorithm in Excel. The half-life of HVA1 and tubulin was estimated using equations derived from the linear regression analysis.

To determine whether HVA1 promotes LR initiation and/or elongation, roots of seedlings treated with 0.2 μM ABA were examined. The number of LRPs was reduced but that of LRs increased in root regions 5.5–7.0 cm and 3.0–4.5 cm from root tips in transgenic lines as compared with WT, indicating that the majority of LRPs initiated were elongated in transgenic lines (Figure 4a,b). The number of LRPs and elongated LRs were both greater in region 0–1.5 cm from the root tip in transgenic lines than in WT (Figure 4a,b).

3xABRC321:HVA1 confers abiotic stress tolerance and promotes WUE in transgenic rice without apparent yield penalty

The tolerance to various abiotic stresses in transgenic rice was tested. With prior acclimation with 150 mM NaCl for 1 week, Tainung 67 transgenic lines displayed higher tolerance to 250 mM NaCl than WT, with survival rates 65–100% for five transgenic lines and only 5% for WT (Figure 5a). Kitaake was more sensitive to drought than Tainung 67 and used in the drought experiment. Although all plants displayed symptoms of leaf rolling and drying under drought, transgenic leaves recovered better than WT after rewatering, with survival rates 58–100% for six transgenic lines and only 25% for WT (Figure 5b). At 4 °C for 3 days, leaves of all plants rolled and dried, but leaves of Tainung 67 and Kitaake transgenic lines opened rapidly and continued to grow at 28 °C (Figure 5c).

Transgenic Tainung 67 and Kitaake expressing HVA1 were treated with sorbitol to induce root growth, and WUEs were determined. Although transgenic lines consumed higher amounts of water, they produced even more biomass compared with WT and thus had higher WUEs (Figure 6a). The performance of transgenic plants was further evaluated in fields. For three separate growing seasons, grain yields in transgenic lines were virtually the same as WT in irrigated fields (Figures 6b and S3). Kitaake was more sensitive to drought than Tainung 67, and therefore, only Kitaake was evaluated in nonirrigated fields. The result indicates that the grain yield in transgenic Kitaake was generally higher than WT in nonirrigated fields (Figures 6b and S3).

3xABRC321:HVA1 has similar tissue-specific and ABA-inducible expression patterns to the endogenous *LEA3* in transgenic rice roots

The expression pattern of HVA1 and the rice LEA3 in WT and transgenic roots was examined by immunocytochemistry assays using antibodies that recognize HVA1 and LEA3. The accumulation of LEA3 in WT and HVA1 in transgenic rice was barely detectable in roots without ABA, but was significantly increased in endodermis and pericycle (En/P) with ABA induction (Figure 7a and Figure S4a). LEA3 and HVA1 were also detected in LRP and in stele and RAM, and levels enhanced by ABA (Figure 7a and Figure S4a). The similarity in expression patterns between HVA1 and LEA3 is likely due to sharing of three cis-acting elements in promoters (Figure S4b). HVA1 was also detected in the cortex and exodermis overlaying the developing LRP in WT and transgenic lines treated with ABA if the antibody fluorescence signals were enhanced (Figure 7b and Figure S5).

The expression pattern of *3xABRC321:GUS* was examined in transgenic roots. GUS was barely detectable without ABA, but was detected at high levels mainly in En/P, founder cells of LRP and RAM after ABA induction (Figure 7c,d). GUS was also detected in the cortex and exodermis overlaying the developing LRP, and LR growth emerged through the

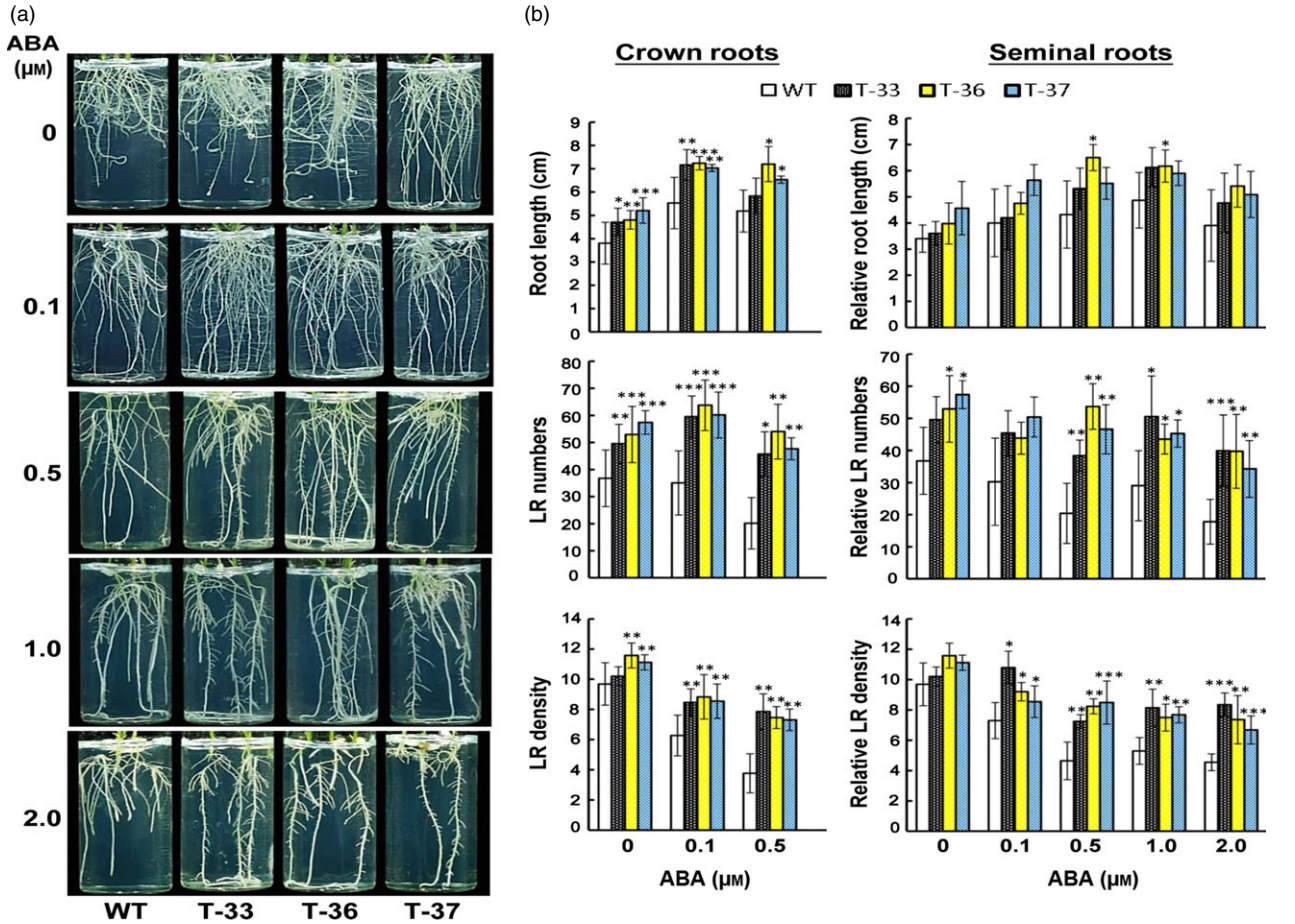

Figure 2 *3xABRC321:HVA1* enhances root growth in transgenic rice under low ABA concentrations. Three-day-old seedlings of three Tainung 67 (T) transgenic lines expressing *3xABRC321:HVA1* were transferred to MS medium containing various concentrations of ABA for 14 days. (a) Root morphology. (b) Relative root length, LR number and LR density of seminal root (right panel) and crown root (left panel) were determined. Error bars represent SD (n=9). Significance levels with the *t*-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Relative root length and LR number of seminal roots mean length of roots elongated and root developed, respectively, after transfer to medium containing various concentrations of ABA.

centre of the GUS-expressing cortex–exodermis region (Figure 7c, d).

3xABRC321:HVA1 and ABA promote root growth in transgenic rice through an auxin-dependent process

The amount and direction of auxin flow in the root determine both the positioning and frequency of LR initiation (Himanen *et al.*, 2002; Orman-Ligeza *et al.*, 2013). As HVA1 and low concentrations of ABA promote the initiation of LRP (Figure 4), we first determined whether auxin is necessary for the HVA1- and ABA-induced root growth. Seedlings were treated with ABA plus or minus the polar auxin transport inhibitor N-(1-naphthyl) phthalamic acid (NPA) (Reed *et al.*, 1998). As compared with the WT, without any treatment, crown roots and LRs were more abundant in transgenic lines (Figure 8a), and with 0.2 μM ABA, seminal and crown roots were significantly longer and LRs were more abundant in transgenic lines (Figure 8b). With NPA, the growth of all types of roots was inhibited (Figure 8c). With both ABA and NPA, the growth of crown roots and LRs was still inhibited, but that of seminal roots continued to extend, with length twice longer than that with NPA alone in transgenic lines (Figure 8d).

PIN-FORMED (PIN) proteins are auxin efflux transporters, and auxin gradient in roots, which depends on the PIN-driven polar auxin transport, is required for the establishment of LRP in both dicots and monocots (Benkova *et al.*, 2003; Petrasek and Friml, 2009). The expression of PINs and a few other proteins that are known to be related to root growth was then examined. The accumulation of *PIN1b*, *PIN2* and *PIN9* mRNAs was higher in transgenic roots than in WT and was all further enhanced by ABA (Figure 8e). Levels of mRNA of three cell cycle regulatory genes, *CYCB1.1*, *CYCB2.1* and *CYCB2.2*, and the auxin-inducible *Crown Rootless 1* (*CRL1*) essential for crown root and LR growth in rice (Inukai *et al.*, 2005) were also higher and further enhanced by ABA in roots of transgenic lines (Figure 8e).

3xABRC321:HVA1 and ABA enhance asymmetrical localization of auxin in LRP founder cells and auxin accumulation in RAM in transgenic rice

DR5:GUS is a widely used marker for estimating endogenous auxin levels (Ulmasov *et al.*, 1997). To determine whether the local distribution of auxin is affected by ectopic expression of HVA1 or by ABA, WT rice and transgenic rice carrying 3*XA*-

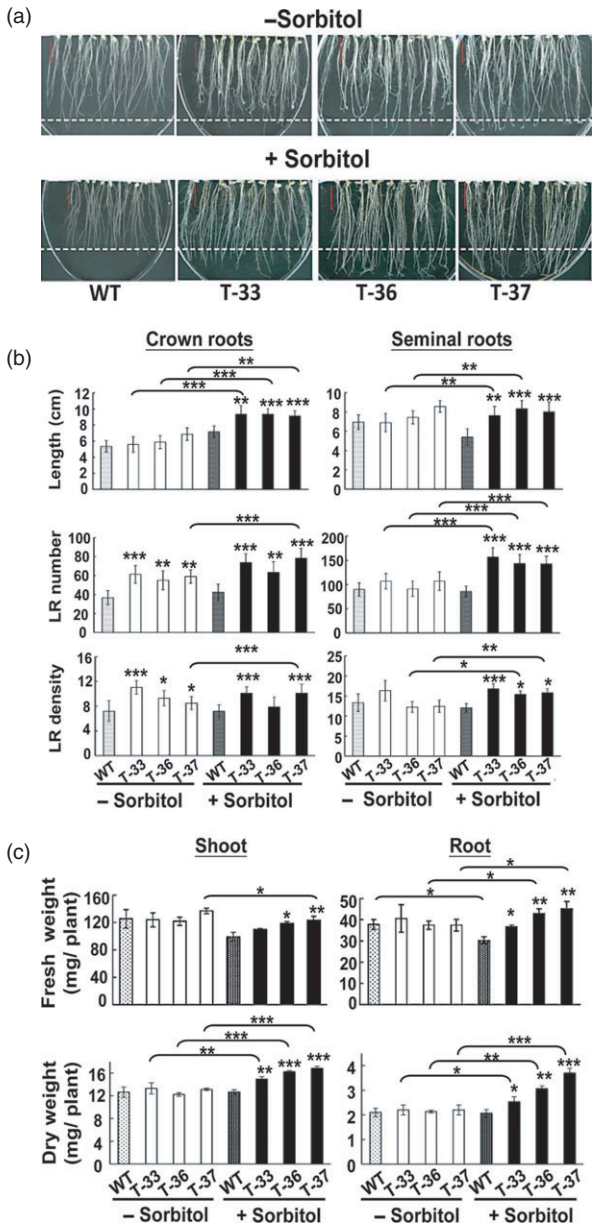

Figure 3 *3xABRC321:HVA1* enhances root growth in transgenic rice under osmotic stress. Three-day-old seedlings of three Tainung 67 (T) transgenic lines expressing *3xABRC321:HVA1* were transferred to MS medium containing 400 mM sorbitol for 11 days. (a) Root morphology. (b) Root length, LR number and LR density of crown roots (left panel) and seminal roots (right panel) were determined. The white dashed line indicates the position of root tips of most plants in WT. (c) Dry and fresh weights of shoots and roots of seedlings in (b) were determined. Error bars represent SD (n = 12). Significance levels with the t-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

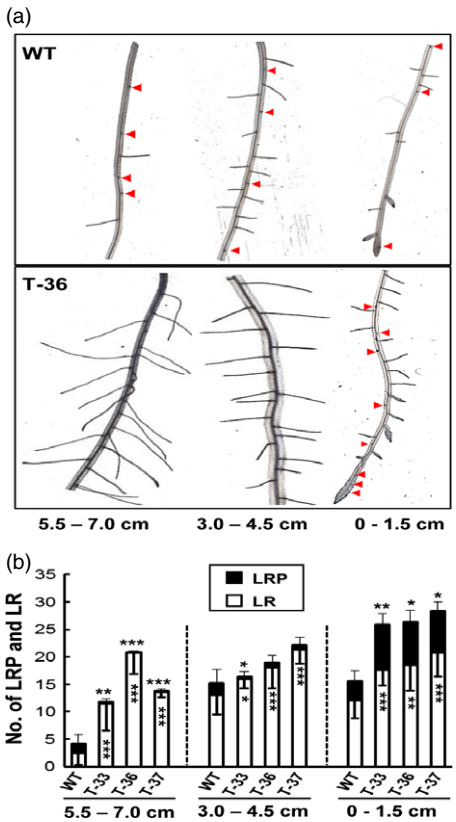

Figure 4 *3xABRC321:HVA1* enhances root initiation and elongation in transgenic rice treated with ABA. Three-day-old seedlings of three Tainung 67 (T) transgenic lines expressing *3xABRC321:HVA1* were transferred to MS medium containing 0.2 μM ABA. (a) Crown roots were treated with 75% ethanol, and LRP and extended LR in three segments (0–1.5 cm, 3.0–4.5 cm and 5.5–7.0 cm from the root tip) were examined. Upper panel: wild type. Lower panel: transgenic line T-36. Arrowhead indicates LRP. (b) The number of LRP and extended LR in (a) was quantified. Error bars represent SD (n = 12). Significance levels with the t-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

BRC321:HVA1 were further transformed with the *DR5:GUS* construct, generating transgenic lines *DR5:GUS* and *HVA1 DR5:GUS*. In crown roots without ABA, GUS expression in both lines was visible along the En/P and at the founder cells of LRP and LR and in RAM (Figures 9a,b, S6a and S7). With ABA, GUS expression in line *DR5:GUS* was reduced or became hardly detectable, but was enhanced in line *HVA1 DR5:GUS* (Figures 9a, b, S6b and S7). The asymmetrical accumulation of GUS in LR founder cells was significantly reduced in line *DR5:GUS*, but was particularly high in line *HVA1 DR5:GUS* with ABA (Figure 9c and Figure S8).

Discussion

A major challenge in modern agricultural biotechnology is designing a transformation cassette enabling the precise temporal and spatial expression of transgene expression at appropriate levels to facilitate growth and enhance abiotic stress tolerance in crops. In this study, we demonstrated that *3xABRC321* functions as a molecular switch to keep low basal level of HVA1 under normal growth conditions, but confers a high level of expression upon induction by ABA, drought, salt and cold stresses in transgenic rice. Moreover, the expression of *3xABRC321:HVA1* successfully improves root architecture and enhances tolerance to various abiotic stresses and WUE in transgenic rice with no yield penalty in irrigated fields and higher yield in nonirrigated field as compared with the WT (Figure 6b).

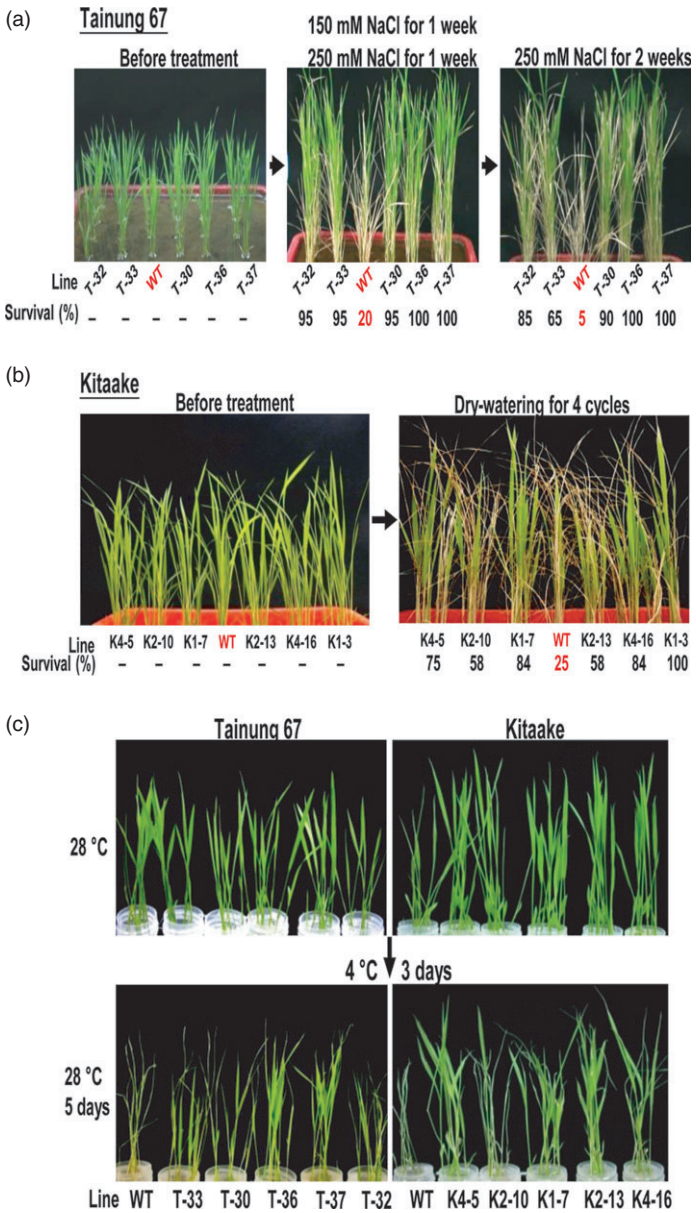

Figure 5 *3xABRC321:HVA1* confers abiotic stress tolerance. (a) Ten-day-old seedlings of Tainung 67 transgenic lines grown in soil were treated with 150 mM NaCl for 1 week and then 250 mM NaCl for 3 weeks. (b) Ten-day-old seedlings of Kitaake transgenic lines grown in soil were subjected to four cycles of complete dry (dehydration) and rewatering (wetting) treatments. Twenty seedlings per line were used for each treatment, and survival rates were determined by counting number of plants that grew when experiments were terminated in (a) and (b). (c) Ten-day-old seedlings of Tainung 67 (left panel) and Kitaake (right panel) transgenic lines grown in hydroponic solution at 28 °C were transferred to 4 °C incubator for 3 days and then shifted back to 28 °C for another 5 days. Survival rates were determined by counting number of plants that grew when experiments were terminated. Twenty seedlings per line were used for each treatment.

Transgenic rice and bent grass overexpressing HVA1 maintain higher leaf relative water content (RWC) and exhibit significantly less leaf wilting than WT and bent grass under drought stress (Babu *et al.*, 2004; Fu *et al.*, 2007). Transgenic mulberry overexpressing HVA1 also shows higher plasma membrane stability than wild type under salinity or water stress (Lal *et al.*, 2008). We observed enhanced expression of three cell cycle regulatory genes by ABA in roots of HVA1 transgenic lines (Figure 8e). These studies suggest that HVA1 transgenic rice may have greater water-holding capacity and RWC, thus be able to maintain photosynthesis and/or metabolic functions under drought conditions. All these metabolic activities eventually led to more active transpiration and growth, contributing to increased total biomass under water stress conditions.

The increase in root growth in HVA1 transgenic rice treated with ABA was resulted from the enhancement of LR initiation and elongation (Figure 4), and the process was auxin dependent based on two observations. First, the HVA1- and ABA-promoted root elongation and LR growth in transgenic rice were inhibited by the auxin transport inhibitor NPA (Figure 8a,b,c,d). Second, the expression of genes encoding several auxin transporters, auxin-dependent cell cycle regulators and positive regulator of crown and LR growth in rice was all increased in transgenic rice and levels further enhanced by ABA (Figure 8e). In the presence of ABA, seminal roots overexpressing HVA1 were less sensitive to NPA inhibition (Figure 8d), suggesting that HVA1 promotes seminal root elongation even with reduced endogenous auxin levels.

In Arabidopsis, auxin is considered a key inducer, while ABA acts as an repressor of LR development (Peret *et al.*, 2009). However, physiological concentrations of ABA seem to induce LRP, as an ABA-insensitive mutant, *abi3*, has a reduced LRP formation in the presence of auxin (Brady *et al.*, 2003). In maize, ABA accumulates rapidly in roots under water stress, and an increase in ABA levels at the root tip is necessary for maintaining PR elongation at low water potentials (Yamaguchi and Sharp,

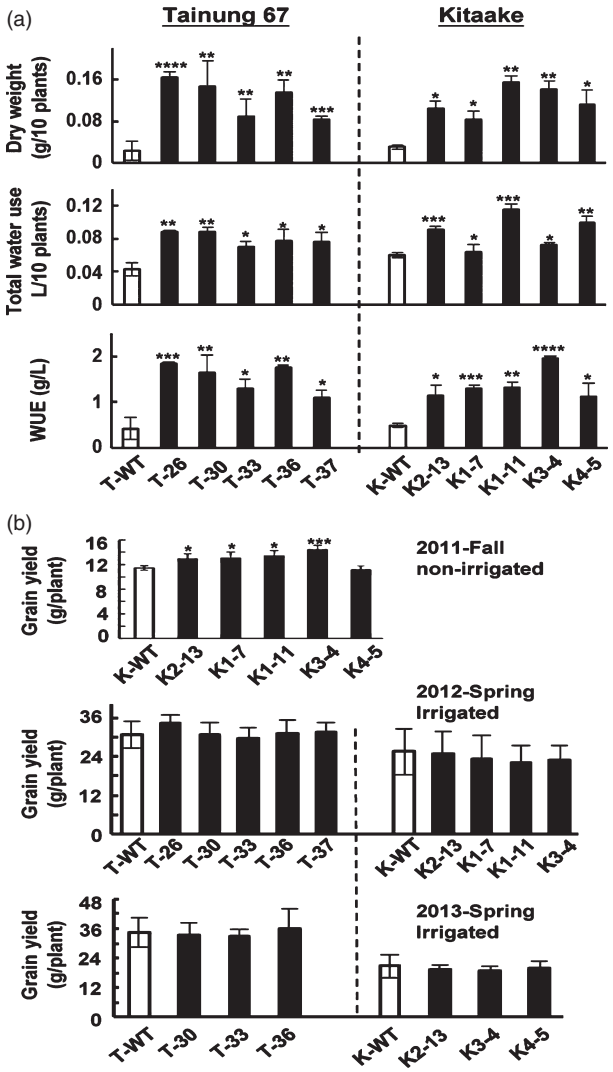

Figure 6 *3xABRC321:HVA1* promotes WUE in transgenic rice without apparent yield penalty. (a) Three-day-old seedlings of Tainung 67 (T) and Kitaake (K) transgenic plants were treated with two cycles of 250 mM sorbitol (3 days) and water (5 days) to induce root growth. Seedlings were then grown in Yoshida solution and transferred to the same amount of fresh solution every 2 days. Total water consumption was measured up to 19 days. Whole-plant dry weights, with two sets of same numbers (n = 12) of plants, before and after 19 days were measured. Upper panel: increased plant dry weights. Middle panel: total water use. Lower panel: WUE determined by dividing total increased plant dry weight by total water use. Error bars represent SD. (b) Tainung 67 (T) and Kitaake transgenic lines were grown in nonirrigated fields in the fall (August to December) of 2011 and in irrigated fields in the spring (March to July) of 2012 and 2013. Grain yield was determined after harvest. Error bars represent SD (n = 10). Significance levels with the t-test: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

2010). In rice, the effect of ABA on root growth appears to be concentration dependent. A low concentration of ABA, 0.1 μM, has been shown to induce root development with enhanced PR elongation and higher root hair density in rice and Arabidopsis (Xu *et al.*, 2013). We also found that 0.1 μM ABA enhanced PR elongation and LR growth in both WT and HVA1 transgenic line (Figure 2). Interestingly, 0.2 μM ABA repressed PR elongation and LR growth in WT but enhanced those in HVA1 transgenic line (Figure 4 and compare Figure 8a with Figure 8b). ABA concentrations higher than 0.5 μM inhibited crown root and LR growth, but less inhibitory to seminal root elongation, and enhanced root thickness in both WT and HVA1 transgenic rice (Figure 2). Hence, ABA treatment may mimic low water potentials or osmotic stress in dehydrated soils, with low concentrations inducing entire root system for increasing root surface for more efficient water uptake, while high concentrations repressing branched roots and promoting root thickness and thus facilitating downwards root growth.

The growth of LRs and PRs was increased in transgenic rice without any hormone or stress treatment (Figure 2). Neither typical auxin response element is found in *ABRC321* nor treatment of 2,4-D or IAA activates *3xABRC321:GUS* expression in transgenic rice. It is likely that the endogenous ABA activates *3xABRC321* to confer low level of HVA1 expression in roots to initiate LRP and PR growth. Exogenous application of low concentrations of ABA or sorbitol elevated the expression of *3xABRC321:HVA1*, leading to more significant increases in root growth.

For a long time, LEAs have represented an enigma in plant biology. The lack of similarity to other proteins of known functions and their high structural flexibility has hampered the research progress regarding the mechanism by which LEAs impact plant growth under abiotic stresses. LEA3 has been shown to be necessary for lateral root growth in Arabidopsis (Salleh *et al.*, 2012). In the present study, we observed that HVA1 promotes the accumulation of auxin in LRP and RAM in the presence of low concentrations of ABA. The auxin-responsive reporter *DR5:GUS* revealed that the asymmetrical accumulation of the auxin maxima at the founder cells of LRP and in RAM was suppressed by 0.2 μM ABA in transgenic line *DR5:GUS* but enhanced in line *HVA1 DR5:GUS* (Figure 9). This result is consistent with the induction of PR and LR growth by 0.2 μM ABA in HVA1 transgenic lines but repression in WT (Figure 8b). The expression of *3xABRC321:GUS* and *DR5:GUS* was partially co-localized in tissues essential for LR and PR growth (Figures 7 and 9), indicating that the ABA-induced *3xABRC321:HVA1* expression plays a positive role in determining the positioning and frequency of LR initiation by altering the amount and/or transport direction of auxin.

The asymmetric accumulation of auxin during plant development could be regulated at the level of biosynthesis, degradation, metabolism or transport (Petrasek and Friml, 2009). PINs are localized in the plasma membrane (Benkova *et al.*, 2003). As aforementioned, HVA1/LEA3 may stabilize proteins or maintain the functional membrane structure under abiotic stresses. Consequently, HVA1 may stabilize PINs in the presence of ABA or under abiotic stress. Alternatively, HVA1 may modulate the auxin homeostasis, for example biosynthesis, metabolism and transport, by stabling important proteins involved in these processes. This notion is supported by the result showing that the accumulation of *PIN1b*, *PIN2* and *PIN9* mRNAs was higher in transgenic roots than in WT, and levels of all of these mRNAs were further enhanced by ABA (Figure 8e).

It is interesting to note that *LEA3*, *3xABRC321:HVA1* and *3xABRC321:GUS* were all expressed in cortical and exodermal cells overlaying the developing LRP (Figure 7b,c,d), indicating that a local inductive signal was transmitted to cells involved in the early commitment stage of LR development. These cells seem to precisely guide and facilitate the LR to push through several cell layers and emerge out of the root surface. These studies

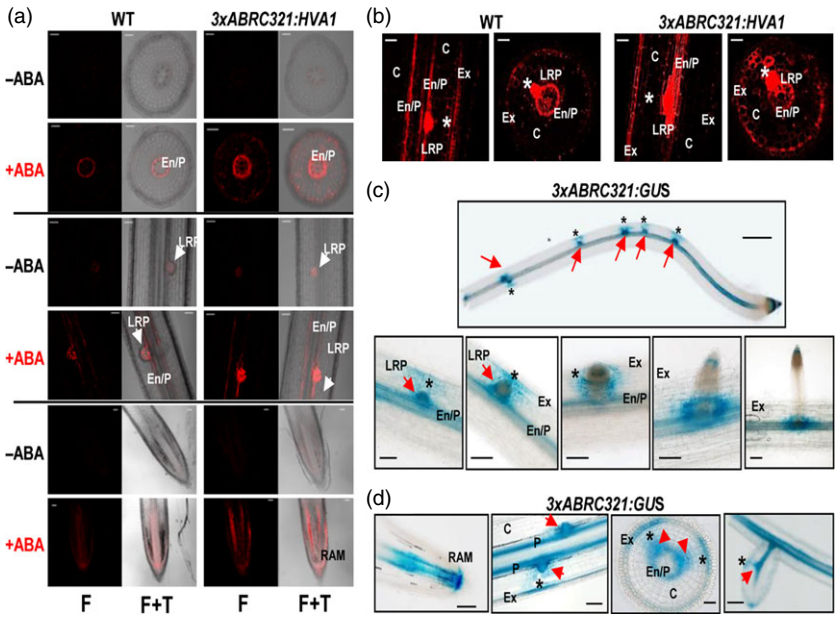

Figure 7 *3xABRC321:HVA1* has similar tissue-specific and ABA-inducible expression patterns to the endogenous *Lea3* in transgenic rice roots. (a) Ten-day-old seedlings of *3xABRC321:HVA1* transgenic line (T-33) were treated with or without 10 μM ABA for 16 h, and roots were sectioned for immunohistological fluorescence assay of LEA3 and HVA1. F: image of fluorescence field. F+T: composite images of fluorescence and transmission fields. For quantitative data of image intensity, see also Figure S4a. (b) Fluorescence images of root longitudinal and cross sections of WT and transgenic lines as prepared in (a) showing expression of LEA3 and HVA1 in cortex and exodermal cells overlaying the LRP as indicated by the asterisk. The image of fluorescence has been enhanced. For original images, please see also Figure S5. (c) Three-day-old seedlings of transgenic lines were treated with 0.2 μM ABA for 11 days, and roots were stained for GUS expression. (d) Roots in (c) were sectioned and stained for GUS expression. Asterisk indicates that GUS was also expressed in cortex and exodermal cells overlaying the LRP in (c) and (d). Arrows indicate LRP. Scale bars: 100 μm. Abbreviation: C: cortex; En: endodermis; Ex: exodermis; P, pericycle.

demonstrated the multiple roles of HVA1 and ABA in promoting LR initiation, elongation and emergence, likely through distinct regulatory pathways.

In summary, our studies demonstrate that *3xABRC321* is an ideal promoter for introducing HVA1 into breeding programs to improve cereals and possibly dicot crops for improvement of tolerance to a wide array of abiotic stresses without an apparent yield penalty. Future studies on mechanisms that regulate the crosstalk between ABA and auxin signals and the function of HVA1/LEA3 in regulating auxin accumulation and/or transport and root growth should facilitate engineering of root architecture for sustainable crop production under stressful environments.

Experimental procedures

Plant materials

Rice cultivars *Oryza sativa* L. cv Tainung 67 and *Oryza sativa* L. cv Kitaake were used for all experiments. Plasmid was introduced into *Agrobacterium tumefaciens* strain EHA101, and rice transformation was performed as described (Chen *et al.*, 2002). Homozygous transgenic lines were used in all experiments. For observation of root growth, transgenic rice seeds were germinated on the surface of half-strength MS medium without sugar but with or without ABA or sorbitol. For stress treatment, transgenic seeds were germinated in water for 5 days and transferred to soil unless otherwise indicated. For hydroponic culture, Yoshida solution was used. Seedlings were normally grown in 28 °C incubator with 12-h daily light.

Plasmids

Plasmid pAHC18 contains the luciferase (*Luc*) cDNA fused between the *Ubi* promoter and the *Nos* terminator (Bruce *et al.*, 1989). Plasmid MP64 contains the barley *Amy64* minimal promoter (−60 relative to the transcription start site) and its 5′ untranslated region (+57 relative to the transcription start site), *HVA22* intron1–exon2–intron2, the *GUS* coding region and the *HVA22* 3′ untranslated region (Shen and Ho, 1995). Plasmid QS115 contains a copy of *HVA22* ABRC1 fused upstream of the *Amy64* minimal promoter in plasmid MP64 (Shen and Ho, 1995).

Plasmid construction

Two 56-bp complementary oligonucleotides containing the CE3 and A2 elements from the *HVA1* promoter and the CE1 element from the *HVA22* promoter (Shen *et al.*, 1996) and restriction sites *Kpn*I, *Xho*I and *Xba*I were synthesized, annealed and designated as *ABRC321* (Figure 1a). *ABRC321* was self-ligated in two or three copies in correct orientations. For expression of GUS under the control of *ABRC321*, 1–3 copies of *ABRC321* were inserted into the *Xba*I site in MP64, so that the *ABRC321* was fused upstream of the barley *Amy64* minimal promoter, generating constructs *1xABRC321-GUS*, *2xABRC321-GUS* and *3xABRC321-GUS* (Figure S1a). For expression of *HVA1* under the control of *3xABRC321*, *GUS* cDNA in construct *3xABRC321-GUS* was replaced with *HVA1* cDNA, generating construct *3xABRC321-HVA1* (Figure S1b).

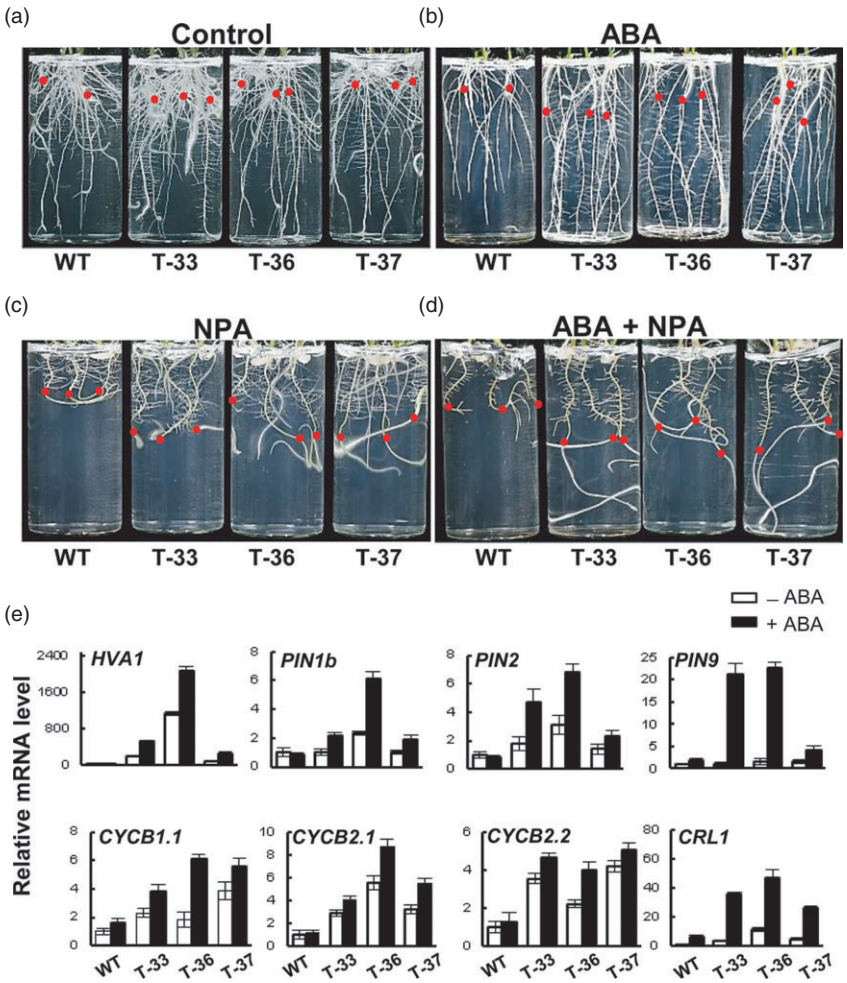

Figure 8 *3xABRC321:HVA1* and ABA promote root growth in transgenic rice through an auxin-dependent process. Three-day-old seedlings of *3xABRC321:HVA1* transgenic line (T-33) were transferred to MS medium containing ABA and/or NPA for 11 days, and root morphology was examined. (a) Medium only, (b) 0.2 μM ABA only, (c) 1 μM NPA only and (d) 0.2 μM ABA plus 1 μM NPA. Red dots indicate starting points of root growth after transferring to medium containing ABA and/or NPA. (e) mRNAs from roots of seedlings prepared in (b) were subjected to quantitative real-time RT-PCR analysis of indicated genes.

GUS activity staining

Sections of leaf and root from 10-day-old seedlings were cut with Microslicers DTK-1000 (TED PELLA, Inc., Redding, CA), incubated in water containing or lacking 10 μM ABA at 28 °C for 24 h and subjected to histochemical staining with a buffer (0.1 M $NaPO_4$, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.5 mM potassium ferricyanide, pH 7.0, and 1 mM X-glucuronide) at 37 °C as described (Jefferson, 1987). After GUS staining, leave samples were incubated in 70% ethanol at 65 °C for 1 h to remove chlorophyll.

Immunohistological fluorescence staining

Tissue localization of HVA1 was examined by modification of a described method (Long *et al.*, 2006). Rice roots were fixed with 2% paraformaldehyde (w/v) in 0.1 M $NaPO_4$ buffer, pH 7.0, and then embedded in 5% agar. Sections were sliced to 30 μm thickness using Microslicers DTK-1000 and incubated in PBS buffer containing 0.3% (v/v) Triton X-100 (PBS-T). The nonspecific reaction was blocked with 5% (w/v) bovine serum albumin in PBS-T. Samples were then incubated with purified rabbit antibarley HVA1 polyclonal antibodies and subsequently with secondary antibodies (Alexa Fluor 555 goat anti-rabbit IgG; Molecular Probes). Samples were examined with a laser scanning confocal microscope (LSM510 META; Zeiss).

Measurement of roots

Rice branched roots could be classified into three types (Supplementary Fig. 3a): seminal roots, also called radical, are the first root emerging during germination; crown roots emerge from the node of coleoptile; and lateral roots branch from the above two types of roots and can bear additional large or small lateral roots until the fifth order of branching. The number of each type of roots was measured by simple counting. Root length was measured, the number of roots was counted, and lateral root density was determined by dividing lateral root number by root length. Error bars represent SD (n = 12). Significance levels with the t-test are as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Dehydration of seedlings

For dehydration tests, 10-day-old seedlings were planted in small pots containing 330 g of soil, grown in a 28 °C chamber and slowly air-dried for 6 days. Pots were then transferred to a bigger container and rewater until soil was completely submerged in water.

Field trial

To evaluate grain yield and biomass production in the field, 25-day-old seedlings were transplanted to a field with 25 × 25 cm of space between each plant. The irrigated field was flooded with

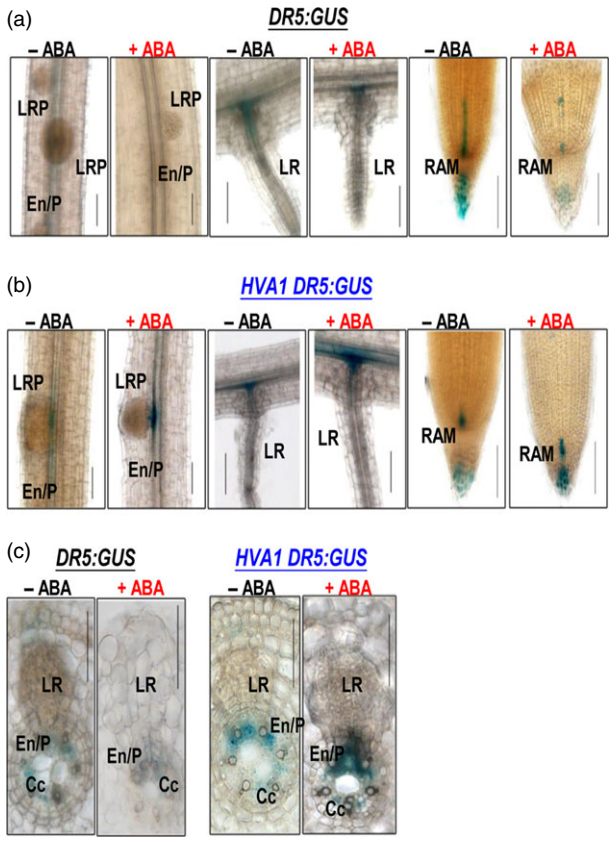

Figure 9 *3xABRC321:HVA1* and ABA enhance asymmetrical localization of auxin in LRP founder cells and auxin accumulation in RAM in transgenic rice. Three-day-old seedlings of transgenic lines *DR5:GUS* and *HVA1 DR5:GUS* were transferred to MS medium with (+) or without (–) 0.2 μM ABA for 11 days, and roots were stained for GUS expression in LR founder cells and RAM, treated with ethanol and examined under a microscope. (a) GUS expression in line *DR5:GUS* was suppressed by ABA. (b) GUS expression in line *HVA1 DR5:GUS* was enhanced or maintained with ABA treatment. For more images of LR founder cells and RAM stained with GUS, see also Figures S6 and S7. (c) Asymmetrical localization of GUS was suppressed in line *DR5:GUS* but enhanced or maintained in line *HVA1 DR5:GUS* with ABA treatment. Scale bar: 100 μm. For images of entire root cross sections, see also Figure S8. Abbreviation: C: cortex; Cc: companion cells; En: endodermis; Ex: exodermis; P, pericycle.

1–5 cm of water (soil water content 37%, v/v) until the end of active tillering stage (30–40 days after transplanting). Water was then drained (soil water content 27%, v/v) for 10–15 days at late tillering stage and flooded again with 3–10 cm of water until the milky stage. Soil in the nonirrigated field was kept moist (soil water content 20~25%, v/v) by intermittent irrigation during the entire planting period. Soil water content was measured using a Theta probe and meter (models ML2x and HH1, Delta-T devices, Cambridge, UK) (Ji *et al.*, 2012). Seeds were harvested, dried and yield determined.

Primer

Nucleotide sequences of primers are listed in Table S1.

Acknowledgements

We thank AndreAna Pena for critical reviewing of the manuscript and Kuo-Wei Lee, Chun-Hsien Lu, Lin-Chih Yu, Ding-Hua Lee and Fang-Min Wu for technical assistance. This work was supported by grants (94F005-1 and 94S1502 to Su-May Yu and 94F005-2 to Tuan-Hua David Ho) from Academia Sinica and NSC101-2321-B001-035 (to Su-May Yu) and NSC102-2321-B-001-048- from the National Science Council of the Republic of China and in part by the Ministry of Education, R.O.C. under the ATU plan to Su-May Yu and Tuan-Hua David Ho.

References

Ahuja, I., de Vos, R.C., Bones, A.M. and Hall, R.D. (2010) Plant molecular stress responses face climate change. *Trends Plant Sci.* **15**, 664–674.

Babu, R.C., Zhang, J., Blum, A., Ho, D.T.-H., Wu, R. and Nguyen, H.T. (2004) HVA, a LEA gene from Barly confers dehydration tolerance in transgenic rice (Oryza sativa L.) via cell membrane protection. *Plant Sci.* **166**, 855–862.

Baena-Gonzalez, E., Rolland, F., Thevelein, J.M. and Sheen, J. (2007) A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**, 938–942.

Bartels, D. (2001) Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance? *Trends Plant Sci.* **6**, 284–286.

Battaglia, M. and Covarrubias, A.A. (2013) Late Embryogenesis Abundant (LEA) proteins in legumes. *Front. Plant Sci.* **4**, 190.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. and Friml, J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591–602.

Brady, S.M., Sarkar, S.F., Bonetta, D. and McCourt, P. (2003) The ABSCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in Arabidopsis. *Plant J.* **34**, 67–75.

Bruce, W.B., Christensen, A.H., Klein, T., Fromm, M. and Quail, P.H. (1989) Photoregulation of a phytochrome gene promoter from oat transferred into rice by particle bombardment. *Proc. Natl Acad. Sci. USA* **86**, 9692–9696.

Chen, P.-W., Lu, C.-A., Yu, T.-S., Tseng, T.-H., Wang, C.-S. and Yu, S.-M. (2002) Rice alpha-amylase transcriptional enhancers direct multiple mode regulation of promoters in transgenic rice. *J. Biol. Chem.* **277**, 13641–13649.

Coudert, Y., Perin, C., Courtois, B., Khong, N.G. and Gantet, P. (2010) Genetic control of root development in rice, the model cereal. *Trends Plant Sci.* **15**, 219–226.

De Smet, I., Zhang, H., Inze, D. and Beeckman, T. (2006) A novel role for abscisic acid emerges from underground. *Trends Plant Sci.* **11**, 434–439.

Dubouzet, J.G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E.G., Miura, S., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J* **33**, 751–763.

Dure, L. (1992) The LEA proteins of higher plants. In *Control of Plant Gene Expression*, (Verma, D.P.S., ed), pp. 325–335. Boca Raton, FL: CRC Press.

Dure, L. 3rd. (1993) A repeating 11-mer amino acid motif and plant desiccation. *Plant J.* **3**, 363–369.

Fu, D., Huang, B., Xiao, Y., Muthukrishnan, S. and Liang, G.H. (2007) Overexpression of barley hva1 gene in creeping bentgrass for improving drought tolerance. *Plant Cell Rep.* **26**, 467–477.

Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V. and Wu, R.J. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl Acad. Sci. USA* **99**, 15898–15903.

Himanen, K., Boucheron, E., Vanneste, S., de Almeida Engler, J., Inze, D. and Beeckman, T. (2002) Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* **14**, 2339–2351.

Ho, T.-H.D. and Wu, R. (2004) Genetic engineering for enhancing plant productivity and stress tolerance. In *Physiology and Biotechology Integration for Plant Breeding*, (Nguyen, H.T. and Blum, A., eds), pp. 489–502. New York, NY: Marcel Dekker Inc.

Hong, B., Barg, R. and Ho, T.-H. (1992) Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. *Plant Mol. Biol.* **18**, 663–674.

Hsieh, T.H., Lee, J.T., Charng, Y.Y. and Chan, M.T. (2002) Tomato plants ectopically expressing Arabidopsis CBF1 show enhanced resistance to water deficit stress. *Plant Physiol.* **130**, 618–626.

Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Shibata, Y., Gomi, K., Umemura, I., Hasegawa, Y., Ashikari, M., Kitano, H. and Matsuoka, M. (2005) Crown rootless1, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. *Plant Cell* **17**, 1387–1396.

Jefferson, R.A. (1987) Assaying chimeric genes in plants from gene fusion system. *Plant Mol. Biol. Report* **5**, 387–405.

Ji, K., Wang, Y., Sun, W., Lou, Q., Mei, H., Shen, S. and Chen, H. (2012) Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *J. Plant Physiol.* **169**, 336–344.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* **17**, 287–291.

Lal, S., Gulyani, V. and Khurana, P. (2008) Overexpression of HVA1 gene from barley generates tolerance to salinity and water stress in transgenic mulberry (Morus indica). *Transgenic Res.* **17**, 651–663.

Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., Fukaki, H., Beeckman, T., Bennett, M. and Laplaze, L. (2013) Lateral root development in Arabidopsis: fifty shades of auxin. *Trends Plant Sci.* **18**, 450–458.

Lee, J.T., Prasad, V., Yang, P.T., Wu, J.F., Ho, T.H.D., Charng, Y.Y. and Chan, M.T. (2003) Expression of Arabidopsis CBF1 regulated by an ABA/stress inducible promoter in transgenic tomato confers stress tolerance without affecting yield. *Plant, Cell Environ.* **26**, 1181–1190.

Liu, Y., Zheng, Y., Zhang, Y., Wang, W. and Li, R. (2010) Soybean PM2 protein (LEA3) confers the tolerance of Escherichia coli and stabilization of enzyme activity under diverse stresses. *Curr. Microbiol.* **60**, 373–378.

Long, S.P., Zhu, X.G., Naidu, S.L. and Ort, D.R. (2006) Can improvement in photosynthesis increase crop yields? *Plant, Cell Environ.* **29**, 315–330.

Lopez-Bucio, J., Cruz-Ramirez, A. and Herrera-Estrella, L. (2003) The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* **6**, 280–287.

Lucas, J. (2003) *World food security at risk as crop yields plateau*. http://www.trust.org/item/20131119230754-aynqh/?source=spotlight.

Malamy, J.E. (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell Environ.* **28**, 67–77.

Marunde, M.R., Samarajeewa, D.A., Anderson, J., Li, S., Hand, S.C. and Menze, M.A. (2013) Improved tolerance to salt and water stress in Drosophila melanogaster cells conferred by late embryogenesis abundant protein. *J. Insect Physiol.* **59**, 377–386.

Moons, A., De Keyser, A. and Van Montagu, M. (1997) A group 3 LEA cDNA of rice, responsive to abscisic acid, but not to jasmonic acid, shows variety-specific differences in salt stress response. *Gene* **191**, 197–204.

Morran, S., Eini, O., Pyvovarenko, T., Parent, B., Singh, R., Ismagul, A., Eliby, S., Shirley, N., Langridge, P. and Lopato, S. (2011) Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. *Plant Biotechnol. J.* **9**, 230–249.

Negrao, S., Courtois, B., Ahmadi, N., Abreu, I., Saibo, N. and Oliveira, M. (2011) Recent updates on salinity stress in rice: from physiological to molecular responses. *Crit. Rev. Plant Sci.* **30**, 329–377.

Nguyen, T. and Sticklen, M.. (2013) Barley HVA1 gene confers drought and salt tolerance in transgenic maize (Zea Mays L.). *Adv. Crop. Sci. Tech.* **1**, 105. doi:10.4172/acst.1000105.

Olvera-Carrillo, Y., Luis Reyes, J. and Covarrubias, A.A. (2011) Late embryogenesis abundant proteins: versatile players in the plant adaptation to water limiting environments. *Plant Signal. Behav.* **6**, 586–589.

Orman-Ligeza, B., Parizot, B., Gantet, P.P., Beeckman, T., Bennett, M.J. and Draye, X. (2013) Post-embryonic root organogenesis in cereals: branching out from model plants. *Trends Plant Sci.* **18**, 459–467.

Peret, B., De Rybel, B., Casimiro, I., Benkova, E., Swarup, R., Laplaze, L., Beeckman, T. and Bennett, M.J. (2009) Arabidopsis lateral root development: an emerging story. *Trends Plant Sci.* **14**, 399–408.

Petrasek, J. and Friml, J. (2009) Auxin transport routes in plant development. *Development* **136**, 2675–2688.

Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J. and Jansen, M.A. (2007) Stress-induced morphogenic responses: growing out of trouble? *Trends Plant Sci.* **12**, 98–105.

Price, A.H., Tomos, A.D. and Virk, D.S. (1997) Genetic dissection of root growth in rice (Oryza sativa L).1. a hydrophonic screen. *Theor. Appl. Genet.* **95**, 132–142.

Reed, R.C., Brady, S.R. and Muday, G.K. (1998) Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiol.* **118**, 1369–1378.

Salleh, F.M., Evans, K., Goodall, B., Machin, H., Mowla, S.B., Mur, L.A., Runions, J., Theodoulou, F.L., Foyer, C.H. and Rogers, H.J. (2012) A novel function for a redox-related LEA protein (SAG21/AtLEA5) in root development and biotic stress responses. *Plant, Cell Environ.* **35**, 418–429.

Shen, Q. and Ho, T.H. (1995) Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel cis-acting element. *Plant Cell* **7**, 295–307.

Shen, Q., Uknes, S.J. and Ho, T.H. (1993) Hormone response complex in a novel abscisic acid and cycloheximide-inducible barley gene. *J. Biol. Chem.* **268**, 23652–23660.

Shen, Q., Zhang, P. and Ho, T.H. (1996) Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. *Plant Cell* **8**, 1107–1119.

Sthapit, B. and Witcombe, J. (1998) Inheritance of tolerance to chilling stress in rice during germination and plumule greening. *Crop Sci.* **38**, 660–665.

Tuong, T.P. and Bouman, B.A.M. (2003) Rice production in water-scarce environments. In *Water Productivity in Agriculture: Limits and Opportunities for Improvement*(Kijne, J.W., Barker, R. and Molden, D., eds), pp. 53–67. UK: CABI Publishing.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K. and Yano, M. (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nat. Genet.* **45**, 1097–1102.

Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T.J. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**, 1963–1971.

Xu, D., Duan, X., Wang, B., Hong, B., Ho, T. and Wu, R. (1996) Expression of a Late Embryogenesis Abundant Protein Gene, HVA1, from Barley Confers Tolerance to Water Deficit and Salt Stress in Transgenic Rice. *Plant Physiol.* **110**, 249–257.

Xu, W., Jia, L., Shi, W., Liang, J., Zhou, F., Li, Q. and Zhang, J. (2013) Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytol.* **197**, 139–150.

Yamaguchi, M. and Sharp, R.E. (2010) Complexity and coordination of root growth at low water potentials: recent advances from transcriptomic and proteomic analyses. *Plant, Cell Environ.* **33**, 590–603.

Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. *Annu. Rev. Plant Biol.* **57**, 781–803.

Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1 The synthetic promoter *3xABRC321* has low-background but is highly inducible by ABA in transgenic rice.

Figure S2 Morphology of various branch roots in rice. A 10-day-old seeding of rice cultivar Tainung 67.

Figure S3 *3xABRC321:HVA1* transgenic lines grown in fields.

Figure S4 *3xABRC321:HVA1* has similar tissue-specific and ABA-inducible expression patterns to the endogenous *LEA3* in transgenic rice roots.

Figure S5 *LEA3* and *3xABRC321:HVA1* are expressed in cortical and exodermal cells overlaying the developing LRP in transgenic rice.

Figure S6 *3xABRC321:HVA1* and ABA enhances asymmetrical localization of auxin maxima in LRP founder cells and in RAM in transgenic rice.
Figure S7 *3xABRC321:HVA1* and ABA enhance asymmetrical localization of auxin in LRP founder cells and auxin accumulation in RAM in transgenic rice.
Figure S8 *3xABRC321:HVA1* and ABA enhances asymmetrical localization of auxin in LRP founder cells.
Table S1 Primer list.