Letter to the Editor

An *irew1* mutant intensifies lateral root branching for adaption upon water stress

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Drought is an environmental threat for agriculture [1]. As climate change continues to heat and dry the world, we are forced to breed more water-saving and high-yielding crops [2]. Plants have developed sophisticated mechanisms to use water efficiently. The plant root is the most important organ to absorb water in soil and root system architecture (RSA) and tropisms are critical strategies to improve plant drought tolerance. RSA consists of the primary root (PR) and lateral root (LR); LR formation plays a central role in this adaptive system [3]. The molecular and cellular mechanism of LR development has been described in most detail in *Arabidopsis* [4]. LR development can be divided into two phases – LR initiation and LR emergence – and demonstrates eight stages of development of primordia [5]. Environmental inputs affect LR branching and plants produce various RSAs in response to environmental stimuli [6,7]. For example, in order to use water efficiently, plants alter RSA to grow shallowly or deeply upon environmental stimuli. Shallow RSAs with increased LR branching are good for capturing water from moist soil at the surface, whereas RSAs with increased PR length and steepness, accompanied by reduced LR numbers and branching angles, can be beneficial for the capture of water from soil in the deep moisture layers when the soil surface is dry [8]. Another useful strategy for water efficiency is root hydrotropism, in which roots chase water toward soil moisture in any direction [8–10]. Plants should highly integrate RSA and hydrotropism to survive under water-deprived conditions.

We previously isolated an *Arabidopsis* ‘improved root elongation under water stress 1’ (*irew1*) mutant that noticeably promotes PR elongation, but not root hydrotropism, under water stress medium (WSM) [11]. Interestingly, *irew1* might stimulate the stress hormone abscisic acid (ABA) response; extra application of Ca^{2+} -enhanced PR elongation in *irew1* mutant and ABA biosynthesis-deficient mutants *ABA deficient 1* (*aba1-1*) and *ABA deficient 2* (*aba2-1*), but not in an ABA receptor sextuple mutant *pyr1pyl1pyl2pyl4pyl5pyl8* (112458) under WSM, indicating that Ca^{2+} signaling intersects with the ABA signal transduction pathway for root adaption upon water stress [11]. In this study, we analyzed the characteristics of RSA of an *irew1* mutant that displays enhanced tolerance to water stress.

To examine the characteristics of the *irew1* mutant’s RSA, we assessed the formation and development of lateral root primordia (LRP) and calculated the LR emergence rates in *irew1* mutant seedlings at 7 days after germination. Seeds of *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) wild-type and *irew1* mutant were first surface sterilized with 100% bleach for 3 min, washed five-times with sterile water and then sown on plates containing half-strength Murashige and Skoog medium supplemented with 1% (w/v) sucrose and 0.8% (w/v) agarose. *irew1* is in Col-0 background. Plates were kept at 4°C for 2 days for vernalization, then the materials were grown vertically for 5 days and transferred to half-strength Hoagland’s medium (normal medium, NM) and half-strength Hoagland’s medium containing 0.3% glycerol in a water potential gradient assay for 2 days as described by Miao *et al.* [9]. The plants were placed in growth chambers under dark–light cycles (8 h dark [21°C] and 16 h light [23°C]). LR numbers were calculated on digital images of the plastic square plates by ImageJ 1.40 software [12]. The number of LRP was counted using a Nikon Eclipse E100 microscope. The clearing of *Arabidopsis* roots for LRP calculation was performed according to the protocol described by Malamy and Benfey [13].

Interestingly, *irew1* mutants displayed increased LR emergence rate compared with the wild-type Col-0 in the normal growth condition (NM), although the total number of LRP in the *irew1* mutant was similar to that in Col-0 in NM (Figure 1A–C), indicating that the *irew1* mutant produced more LR than the wild-type using the same amount of LRP. On the other hand, the wild-type Col-0 displayed fewer LR and LRP under WSM and the LR emergence rate under WSM was similar to that of Col-0 in NM. However, the *irew1* mutant exhibited similar numbers of LRP in NM and WSM but produced more LR upon WSM than in NM; thus the *irew1* mutant could access more water and demonstrated a better adaption capability in response to water-deficient conditions (Figure 1C & D).

To further investigate the mechanism of the *irew1* mutant, we extracted total RNA from the roots of *irew1* mutant and wild-type Col-0 seedlings at 7 days after germination in NM and WSM, using the TRIzol reagent (Beijing TransGen Biotech Co., Ltd, Beijing China) in accordance with the manufacturer’s protocol according to Yuan *et al.* [11]. The RNA-Seq data used in the study was deposited in the Gene Expression Omnibus [14] under code PRJNA605515. The number of downregulated root development-related differentially expressed genes for Col-0-NM versus Col-0-WSM was more than that of *irew1*-NM versus *irew1*-WSM, suggesting that *irew1* preserved root

Figure 1. *irew1* mutant sustains the expression of root development-related genes and triggers lateral root branching under water stress. (A & B) The LRP numbers of the wild-type Col-0 and *irew1* mutant grown in NM (A) and WSM (B). (C) The total LRP of the wild-type Col-0 and *irew1* mutant in NM and WSM. (D) The LR emergence rate of the wild-type Col-0 and *irew1* mutant in NM and WSM. The values are means and error bars show $\pm$ SD of 12 seedlings from three independent experiments ($n = 36$). (E) Heat map chart derived from hierarchical cluster analysis of root development-related genes that emerged as altered in RNA-Seq results. Each row represents a gene; columns indicate Col-0-WSM versus Col-0-NM, Col-0-NM versus *irew1*-WSM, *irew1*-NM versus *irew1*-WSM and Col-0-WSM versus *irew1*-WSM, respectively. Red and green indicate increase and decrease, respectively, of the levels of gene expression compared with the unstimulated control, while black indicates no change. (F) Number of root development-related genes in Col-0-WSM versus Col-0-NM, Col-0-NM versus *irew1*-WSM, *irew1*-NM versus *irew1*-WSM and Col-0-WSM versus *irew1*-WSM. Downregulated and upregulated genes are shown in green and red, respectively. Col-0: Columbia-0; LRP: Lateral root primordia; NM: Normal medium; SD: Standard deviation; WSM: Water stress medium.

development-related gene expression under WSM (Figure 1F). In the transcriptome analysis, interestingly, we found that the expression level of *rgf7* (AT3G02240), encoding a root meristem growth factor, was upregulated in *irew1*, but not in Col-0, in response to water stress (Figure 1E). *rgf7* is a member of the secreted peptides family and is expressed mainly in the innermost layer of central columella cells, which are required for conservation of the root stem cell niche and the proliferation of transit-amplifying cells [15,16]. As for *rgf7*, the expression level of *rgf3* (AT2G04025) in *irew1* was also found to be higher than that of Col-0. Conversely, the expression of multiple genes related to LR growth and development was downregulated in Col-0 and unchanged in *irew1* (Figure 1E); such genes included *air1* (AT4G12550), *lrx1* (AT1G12040), *gasa1* (AT1G75750) and *pip2* (AT3G53420). Taken together, RNA-Seq data revealed that the *irew1* mutant optimized the expression profiles of root-related genes and sustained root growth under water stress.

Conclusion

The *irew1* mutant might intensify LR formation to expand the effective water absorption area for root adaption in water-deficient conditions.

Author contributions

S Huang and R Miao carried out all the experiments and the majority of the data analysis. R Miao completed the data analysis, performed statistical analysis and wrote the manuscript.

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