A Nck-associated protein 1-like protein affects drought sensitivity by its involvement in leaf epidermal development and stomatal closure in rice

Lichao Huang1,†, Long Chen1,†, Lan Wang1, Yaolong Yang1, Yuchun Rao1, Deyong Ren1, Liping Dai1, Yihong Gao1, Weiwei Zou1, Xueli Lu1, Guangheng Zhang1, Li Zhu1, Jiang Hu1, Guang Chen1, Lan Shen1, Guojun Dong1, Zhenyu Gao1, Longbiao Guo1, Qian Qian1* and Dali Zeng1***

1State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China, and 2College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua 321004, China

Received 5 November 2018; revised 9 February 2019; accepted 13 February 2019; published online 13 February 2019.

For correspondence (e-mails qianqian188@hotmail.com or dalizeng@126.com).

These authors contributed equally to this article.

SUMMARY

Water deficit is a major environmental threat affecting crop yields worldwide. In this study, a drought stress-sensitive mutant drought sensitive 8 (ds8) was identified in rice (Oryza sativa L.). The DS8 gene was cloned using a map-based approach. Further analysis revealed that DS8 encoded a Nck-associated protein 1 (NAP1)-like protein, a component of the SCAR/WAVE complex, which played a vital role in actin filament nucleation activity. The mutant exhibited changes in leaf cuticle development. Functional analysis revealed that the mutation of DS8 increased stomatal density and impaired stomatal closure activity. The distorted actin filaments in the mutant led to a defect in abscisic acid (ABA)-mediated stomatal closure and increased ABA accumulation. All these resulted in excessive water loss in ds8 leaves. Notably, antisense transgenic lines also exhibited increased drought sensitivity, along with impaired stomatal closure and elevated ABA levels. These findings suggest that DS8 affects drought sensitivity by influencing actin filament activity.

Keywords: drought sensitivity, stomatal closure, abscisic acid, stomatal density, cuticle, rice (Oryza sativa L.).

INTRODUCTION

Adverse climatic conditions, such as drought, threaten rice growth and production. To adapt to drought stress, plants have evolved various mechanisms involving adaptive changes at the morphological, physiological and molecular levels. Characteristics of the leaf epidermis are important indicators of plant drought tolerance (Fang and Xiong, 2015).

In rice, the epidermal cell wall is covered by a hydrophobic cuticle on the outside and embedded with silica beneath the cuticle (Kunst and Samuels, 2003; Ma and Yamaji, 2008). The cuticle, composed of cutin and wax, is an indispensable barrier that prevents non-stomatal water loss (Mao et al., 2012). Mutants with changes in cuticle wax components, such as wsi1, wsi3, wsi4, cfl1 and dwa1, have increased sensitivity to drought stress (Yu et al., 2008; Wu et al., 2011; Zhu and Xiong, 2013; Gan et al., 2016; Wang et al., 2017). The silica-embedded cuticular papillae (CP) also help reduce cuticular transpiration in rice (Yoshida et al., 1962; Agarie et al., 1998; Epstein, 1999; Gao et al., 2005).

Stomata, which play a role in water and gas exchange, are responsible for approximately 90% of the water loss that occurs in the leaf epidermis (Buckley, 2005). Stomatal density and stomatal aperture are therefore important indices used to evaluate drought resistance in plants. Over-expression lines of OsDT11 and SDD1 display dramatically enhanced drought tolerance due to their reduced stomatal densities (Yoo et al., 2010; Li et al., 2016). Stomata generally close in response to low environmental humidity to prevent excessive water loss (Pantin and Blatt, 2018). Genes that positively modulate stomatal closure can improve the survival rates of plants during drought stress (Huang et al., 2009; Jin et al., 2013; Dey et al., 2016; Gao et al., 2018).

Abscisic acid (ABA) induces stomatal closure through a complex signaling network (Munemasa et al., 2015; Matsuda et al., 2016; Sussmilch and McAdam, 2017). The perception of the ABA signal by ABA receptors activates downstream plasma membrane Ca2+ channels and K+ efflux channels, resulting in stomatal closure (Munemasa et al., 2018).
et al., 2015). A decrease in the ABA content in guard cells dampens the response of leaf stomata to ABA stimulation, leading to reduced stomatal closure, followed by increased sensitivity to water loss (Kang et al., 2010; Matsuda et al., 2016). ABA also regulates stomatal aperture by driving reactive oxygen species (ROS) biosynthesis (Mittler and Blumwald, 2015; Sierla et al., 2016). Rice plants with elevated H$_2$O$_2$ levels in guard cells exhibit reduced stomatal aperture and increased tolerance to water deficit (Liu et al., 2016; Hu et al., 2017).

Most studies on ABA-mediated stomatal closure have focused on the functions of ABA receptors or ion channels in guard cells (Park et al., 2009; Geiger et al., 2010; Guo et al., 2011; Zhao et al., 2016; Müller et al., 2017). Some studies have pointed to a relationship between the ABA-signaling network and actin filaments (Li et al., 2014). Actin filaments are major components of the complex cytoskeleton, and participate in various cellular events affecting plant growth and development (McCurdy et al., 2001; Thomas and Staiger, 2014). During stomatal closure, the actin filaments in guard cells undergo a depolymerization-repolymerization process that functions upstream of stomatal movement (Eun and Lee, 2000; Gao et al., 2001; Thomas and Staiger, 2014). To ensure successful stomatal closure, the radially oriented actin filaments in open stomata must depolymerize, become randomly distributed, and reorient in longitudinal actin cables (Dong et al., 2001; Lemichez et al., 2001; Zhang et al., 2007; Higaki et al., 2010; Zhao et al., 2011). Suppressed actin filaments dynamics in Arabidopsis thaliana guard cell delays ABA-mediated stomatal closure and results in hypersensitivity to drought stress (Zhao et al., 2011). The Arp2/3 complex, comprising seven elements (ARP2, ARP3, ARPC1, ARPC2, ARPC3, ARPC4 and ARPC5), is activated by the SCAR/WAVE complex to increase the efficiency of actin filaments nucleation and to initiate new actin filaments branching (Pollard, 2007; Yanagisawa et al., 2015). The SCAR/WAVE complex consists of five evolutionarily conserved subunits, namely ABI, BRICK1/HSPC300, NAP1/NAP125, PIR/SRA1 and SCAR (Le et al., 2006; Mendoza, 2013; Zhou et al., 2016). The SCAR/WAVE complex members can enhance the Arp2/3 complex-mediated nucleation efficiency of actin filaments in vitro and in vivo (Welch and Mullins, 2002; Bai et al., 2015). All those results indicated that the SCAR/WAVE-Arp2/3 complex may be involved in ABA-mediated stomatal closure.

Enormous effort has been devoted to elucidating the role of the SCAR/WAVE-Arp2/3 complex in the formation and development of leaf epidermal cells by involvement in actin filaments modeling over the past decades (Frank and Smith, 2002; Djakovic et al., 2006; Facette et al., 2015); however, few studies have focused on the function of SCAR/WAVE complex in plant responses to environmental stress, especially drought stress. In our study, the Nck-associated protein 1 (NAP1)-like protein coding gene DROUGHT SENSITIVE 8 (DS8) was hypothesized to affect drought sensitivity by influencing actin filament activity. The study was designed to find out how DS8 affects drought sensitivity in rice plant, focusing on the impacts of DS8 on indicators related to drought sensitivity, including cuticle and stoma. Cytochalasin B (CB) and sodium tungstate treatments were performed to preliminarily analyze the relationship between ABA and actin filaments activity during ABA-induced stomatal closure.

RESULTS
ds8 is sensitive to drought stress

The ds8 mutant was screened under drought stress conditions from a Nipponbare (NBP, japonica) rice mutant pool generated by ethyl methanesulfonate mutagenesis. A drought stress test indicated that ds8 seedlings were more sensitive to drought stress than the wild-type (Figure 1a). After recovery from drought treatment, the survival rate of ds8 was only 9.4% that of wild-type (Figure 1b). In addition, when excised flag leaves were placed in an incubator at 60% humidity, ds8 leaves curled quickly (Figure 1c) and showed a higher water loss rate than wild-type leaves (Figure 1d). We also investigated major agronomic traits related to grain yield in dry environmental conditions. Under drought conditions, ds8 suffered higher decline rates in agronomic traits such as tiller number, grain number per panicle, filled grains per panicle and grain yield per plant than the wild-type (Figure S1; Table S1).

Map-based cloning of DS8

Reciprocal crosses between ds8 and three varieties (9311, ZF802 and CJ06) of wild-type rice plants were carried out. Plants from all six F$_1$ populations exhibited normal phenotypes. In all F$_2$ populations, the ratio of wild-type to ds8 mutant phenotypes in every combination was 3:1 (Table S2). We performed map-based cloning, and mapped DS8 to chromosome 8 between markers RM1345 and RM3120 (Figure 2a). The primer sequences used are listed (Table S3). Furthermore, using 2319 homozygous mutant plants, DS8 was narrowed down to a 53.7-kb genomic region between markers E1 and E2. There were seven predicted open reading frames (ORFs) in this region, according to the Rice Genome Annotation Project (RGAP). We sequenced these predicted ORFs and identified a single-base substitution (G to A) at the last nucleotide of the second intron in LOC_Os08g43130 in ds8. This mutation caused a premature stop codon due to the deletion of the entire third exon (81 bp) and part of the 17th exon (8 bp; Figure 2b).

A genetic complementation test was arranged to ds8 mutant and 22 positive proDS8:DS8 transformants were
obtained, all transformants exhibited wild-type levels of drought tolerance, confirming the identity of DS8 as LOC_Os08g43130 (Figures 2c,d and S2). Moreover, the leaf water loss rate of the transgenic plants was similar to that of the wild-type (Figure 2e; Table S4).

We carried out quantitative reverse-transcription polymerase chain reaction analysis of DS8 transcript levels in various tissues at the booting stage. DS8 was widely expressed in panicle, culm, sheath, root and leaf tissue (Figure S3a). We also detected GUS (β-glucuronidase) activity in these tissues in proDS8:GUS transgenic rice plants (Figure S3b–f). Analysis of the subcellular localization of DS8-eGFP in rice protoplasts and pro35S:DS8-eGFP transgenic plants indicated that DS8 was localized to the cytoplasm (Figure S3g,h).

DS8 encodes a NAP1-like protein involved in actin filaments function

Sequence analysis showed that DS8 comprised 23 exons and 22 introns, and encoded a 1359 amino-acid protein (Figure 2b). A protein–protein BLAST search against the NCBI database using the deduced DS8 protein sequence as a query revealed that it was a NAP1-like protein. We selected 25 DS8 homologs from various species to determine the evolutionary relationship between DS8 and these proteins, finding that the DS8 homologs were conserved in both monocots and dicots (Figure S4).

Protein sequence alignment revealed that DS8 shares 86.28% amino acid identity with homologs from Hordeum vulgare, Sorghum bicolor, Zea mays and A. thaliana (Figure S5).

The organization of actin filaments in the seedling roots of ds8 and wild-type plants was observed. The actin filaments were complete and regularly arranged in wild-type. In ds8, disordered actin filaments were observed at the meristematic zone, became more severe during cell growth from the meristematic zone to maturation zone, and appeared incomplete and cluttered in the maturation zone (Figure 2f–h).

DS8 is involved in leaf cuticle development

We stained the leaves of wild-type and ds8 plants with toluidine blue, which can roughly reflect the integrity of the cuticle on the leaf surface. Wild-type leaves successfully resisted toluidine blue staining, whereas ds8 leaves were partially stained by the blue dye (Figure 3a). Transmission electron microscopy (TEM) analysis showed that the cuticular layer of the leaf epidermis in ds8 was significantly thicker (nearly 1.85-fold) than that of wild-type (Figure 3b,c). However, the less osmiophilic membrane of ds8 exhibited reduced electron density, as revealed by its lighter coloring (Figure 3b). We also compared the wax components of the leaf cuticles of wild-type and ds8 plants. The levels of very-long-chain fatty acids,
such as C20:0, C24:0 and C26:0, were reduced in ds8, whereas the levels of alkanes such as C33:0 and C35:0 were significantly higher in ds8 compared with the wild-type (Figure 3d).

The cell wall components in wild-type and ds8 leaves were also measured. Pectin, hemicellulose I and cellulose levels were significantly lower in ds8 leaves than in wild-type leaves (Figure S6a). The levels of monosaccharides such as arabinose, mannose, fructose, glucose and galactose were also significantly reduced in ds8 leaves (Figure S6b).

**DS8 affects stomatal density**

Observation of leaf ultrastructure revealed that the bulliform cell band on the upper leaf epidermis of ds8 was more wrinkled than that of the wild-type (Figure 4a). Cross-sections of ds8 leaves exhibited shrunken bulliform cells, leaving a groove between two vascular bundles (Figure 4b). The width of the S-ST-BC unit in the leaf epidermis of wild-type and ds8 was measured. The width of the S-ST-BC unit in ds8 leaf was reduced to approximately...
64.4% that of the wild-type (Figure 4c). The stomatal density in ds8 leaves showed an obvious increase compared with the wild-type (Figure 4d).

**DS8 affects stomatal closure and abscisic acid accumulation**

The stomatal aperture status on the upper leaf epidermis was examined. In general, the stomatal aperture status was categorized into three types (Figure 5a): completely open (CO); partially open (PO); and completely closed (CC). The proportion of CO stomata in seedling leaf was significantly higher in ds8 than in the wild-type (approximately 86 and 54%, respectively) under field conditions. Meanwhile, the proportions of PO and CC stomata were significantly lower in ds8 than in the wild-type (Figure 5b). We then treated wild-type and ds8 leaves with ABA and \( H_2O_2 \), which can promote stomatal closure. After being opened to a maximum level, the proportion of CO-type stomata was significantly reduced in wild-type leaves during ABA or \( H_2O_2 \) treatment, whereas the degree of CO-type stomata in ds8 leaves exhibited an unexpected increase (Figure 5c). In addition, the ABA levels in wild-type and ds8 leaves during various growth phases in the paddy field were measured. The endogenous ABA content was significantly higher in ds8 than in the wild-type. The ABA content remained high in ds8 leaves throughout the growing season (Figure 5d). Furthermore, the ds8 mutant exhibited withered leaf tips under natural field conditions (Figure S7a,b).

**DS8-antisense transgenic lines exhibit increased drought sensitivity**

The DS8-antisense transgenic lines were generated. Two antisense transgenic lines with clearly reduced DS8 transcript levels showed increased sensitivity to drought stress (Figure 6a,b). Compared with the wild-type, the proportion of CO-type stomata and ABA content were both higher in the DS8-antisense transgenic seedlings under normal field conditions (Figure 6c,d). Excised leaves harvested from
these lines showed an intermediate water loss rate between wild-type and ds8 levels (Figure 6e; Table S5). These results confirmed that DS8 was involved in leaf water loss and foliar ABA accumulation.

**Suppressed actin filaments activity causes increased abscisic acid accumulation**

We treated 13-day-old wild-type seedlings with 5 μM CB, an inhibitor of actin polymerization; 0.05% dimethylsulfoxide (DMSO) treatment was used for the control. Although 0.05% DMSO treatment led to disordered actin filaments to a certain extent, the number of actin filaments in CB-treated root tips was clearly reduced compared with the control (Figure 7a). The ABA content of the seedling leaves was 33.97 ng g⁻¹ on the 4th day after 0.05% DMSO treatment, whereas it was approximately 141.73 ng g⁻¹ after CB treatment (Figure 7b). The foliar ABA content increased when the activity of actin filaments was suppressed.

On the other hand, ds8 seedlings were treated with various concentrations of sodium tungstate, an ABA biosynthesis inhibitor (Figure 7c). The ABA content in ds8 seedling leaves decreased with the increase of sodium tungstate concentration. Unexpectedly, the degree of stomatal closure in ds8 leaves increased in response to decreased ABA content, reaching a maximum when the ABA content was similar to that of wild-type (when the concentrations of sodium tungstate was 1.0 mM). With further decline of ABA content in response to a higher concentration of sodium tungstate (1.5 mM and 2.0 mM), the degree of stomatal closure in ds8 leaves turned back to a decrease (Figure 7d).

**DISCUSSION**

In plants, SCAR/WAVE complex subunits were generally reported to affect cell morphogenesis by regulating actin filaments nucleation and branching. Their functions in drought stress were rarely mentioned (Brembu et al., 2004; Salah et al., 2004; Le et al., 2006; Panteris et al., 2009). In this study, we isolated and characterized the rice mutant ds8, which exhibited increased sensitivity to drought stress. DS8 encoded a NAP1-like protein, a component of the SCAR/WAVE complex. DS8 is reported to encode an interacting protein of LPL2, a PIROGI/Specifically Rac1-associated protein 1 (PIR/SRA1)-like protein involved in...
Figure 5. \(DS8\) affects stomatal closure and abscisic acid (ABA) accumulation.
(a) Three different stomatal aperture types in wild-type (WT; upper panels) and \(ds8\) (lower panels): completely open (CO; left), partially open (PO; middle) and completely closed (CC; right). Scale bar: 5 \(\mu\)m.
(b) Percentage of each type of stoma in WT and \(ds8\). At least 100 stomata from five individual plants were analyzed for WT and \(ds8\), respectively. Comparison was made between WT and \(ds8\) of the same stoma type. Data are represented as mean ± SD. **\(P \leq 0.01\); Student’s t-test.
(c) Percentage of each type of stoma in WT and \(ds8\) during ABA and H\(_2\)O\(_2\) treatment. Leaves with maximum stomatal aperture status were used as the control. At least 100 stomata from five individual plants were analyzed per treatment. Comparison was made between WT and \(ds8\) of the same stoma type in each treatment. Data are represented as mean ± SD. **\(P \leq 0.01\); *\(P \leq 0.05\); Student’s t-test.
(d) Foliar ABA content in WT and \(ds8\) during different growth phases in the paddy field. S, seedling stage; H, heading stage; 15 DAF, 15 days after flowering. Data are represented as mean ± SD (\(n = 3\)). **\(P \leq 0.01\); Student’s t-test.
(e) 3,3’-Diaminobenzidine (DAB) staining of excised leaves from WT (left) and \(ds8\) (right) plants. Scale bar: 1 cm.
(f) Nitrotetrazolium chloride (NBT) staining of excised leaves from WT (left) and \(ds8\) (right) plants. Scale bar: 1 cm.
(g) Catalase (CAT) activity in WT and \(ds8\) leaves. Data are represented as mean ± SD (\(n = 3\)). *\(P \leq 0.01\); Student’s t-test.
(h) H\(_2\)O\(_2\) content in WT and \(ds8\) leaves. Data are represented as mean ± SD (\(n = 3\)). **\(P \leq 0.01\); Student’s t-test.
(i) Comparison of electrolyte leakage between WT and \(ds8\). Data are represented as mean ± SD (\(n = 3\)). **\(P \leq 0.01\); Student’s t-test.
pavement cell morphology. Its T-DNA insertion mutant exhibits similar deficient pavement cell morphology to lpl2 (Zhou et al., 2016). Here, we further cloned and identified DS8 by functional complementation analysis and revealed its function in affecting drought sensitivity.

**DS8 is involved in actin filaments modeling**

In Arabidopsis, GNARLED encodes a NAP1 homolog that is reported to positively regulate the function of SCAR/WAVE-Arp2/3 complex in the process of actin filaments nucleation and branching (Machesky et al., 1999; Deeks et al., 2004; Salah et al., 2004). In this study, DS8 shared high sequence similarity with GNARLED, according to the multiple sequence alignment (Figure S5). Like most SCAR/WAVE subunit mutants, ds8 exhibited an abnormal actin cytoskeleton (Figure 2f–h). These results indicated that DS8 encoded a NAP1 homolog that was involved in the nucleation of actin filaments by contributing to SCAR/WAVE-Arp2/3 complex activity.

**DS8 is involved in cuticle development**

The successful transition of extant land plants from aquatic to terrestrial habitats has largely depended on the evolutionary acquisition of unique adaptations to the terrestrial environment, such as the distinctive epidermal components (Chen et al., 2017). Disorganized actin filaments in plants with SCAR/WAVE complex deficiency results in changes in epidermal trichome (in Arabidopsis)/CP (in rice) and pavement cell morphology (Basu et al., 2004, 2005; Deeks et al., 2004; Djakovic et al., 2006; Bai et al., 2015; Rao et al., 2015; Zhou et al., 2016). In our study, we also found epidermal changes in ds8 plants.

The shrunken bulliform cells (Figure 4b) explained the wrinkled bulliform cell band (Figure 4a) observed on the upper leaf epidermis of the ds8. The wrinkled bulliform cell band decreased the width of the S-ST-BC unit on mutant leaf surface to a certain extent (Figure 4c), which increased the number of S-ST-BC units per unit area and finally increased stomatal density (Figure 4d). So, the increased stomatal density in ds8 due to altered bulliform cell morphology was a byproduct of abnormal epidermal cell morphogenesis.

Unlike other SCAR/WAVE subunit mutants, the cuticle of ds8 leaves was impaired according to the result of toluidine blue staining (Figure 3a). ds8 exhibited altered cuticle wax components (Figure 3d) and cell wall deposition (Figure S6) in leaves. The changes in proportion of cuticular wax components (Figure 3d), which could cause

---

Figure 6. DS8 antisense transgenic lines exhibit increased drought sensitivity.
(a) DS8 antisense transgenic lines subjected to drought stress. Scale bar: 5 cm.
(b) DS8 transcript level in wild-type (WT), ds8 and antisense transgenic lines. Data are represented as mean ± SD (n = 3). Different letters above columns indicate statistically significant differences (least significance difference test, P ≤ 0.05).
(c) Percentage of each type of stoma in WT, ds8 and DS8 antisense transgenic lines. CO, completely open; PO, partially open; CC, completely closed. Data are represented as mean ± SD. At least 100 stomata from five individual plants were analyzed, respectively, in WT, ds8 and each antisense transgenic line. Different letters next to columns indicate statistically significant differences between WT, ds8 and antisense transgenic lines of the same stoma type (least significance difference test, P ≤ 0.05).
(d) Abscisic acid (ABA) content in WT, ds8 and antisense transgenic lines. Data are represented as mean ± SD (n = 3). Different letters above columns indicate statistically significant differences (least significance difference test, P ≤ 0.05).
(e) Water loss rates of excised leaves harvested from WT, ds8 and antisense transgenic plants at the heading stage. Data are represented as mean ± SD (n = 3). The least significance difference test was applied at 0.05 probability level (Table S5).

© 2019 The Authors. The Plant Journal published by John Wiley & Sons Ltd and Society for Experimental Biology.
defects in epicuticular wax (Yu et al., 2008; Qin et al., 2011; Mao et al., 2012; Zhu and Xiong, 2013; Gan et al., 2016; Wang et al., 2017), accounted for the damaged cuticle layer in ds8. Some rice mutants with abnormal cuticles have thicker but less dense cuticular layers when compared with wild-type plants (Mao et al., 2012; Gan et al., 2016), which could also be observed in our study (Figure 3b,c). All these results suggested that DS8 not only functioned in epidermal cell morphogenesis like other SCAR/WAVE subunits, but also influenced the development of the cuticle and cell wall. Therefore, DS8 was important for building complete leaf epidermis to protect plants against non-stomatal water loss.

**DS8 is required for abscisic acid-induced stomatal closure**

Mechanistic studies of stomatal movement have demonstrated that actin filaments dynamics functions downstream of ABA-regulated stomatal movement, and the inhibition of actin filaments nucleation or distribution reduces stomatal closure in response to ABA or H$_2$O$_2$ stimulation (Pei et al., 2000; Zhang et al., 2001; Gudesblat et al., 2007; Hardham et al., 2007; MacRobbie and Kurup, 2007; Gao et al., 2009; Kim et al., 2010; Zhao et al., 2011; Jiang et al., 2012; Li et al., 2014; Munemasa et al., 2015; Cao et al., 2016). So, the presence of abnormal actin filaments in ds8 explained why the mutant leaves exhibited a reduced degree of stomatal closure during normal growth (Figure 5b). The stomatal closure defect in ds8 could not be recovered by exogenous ABA or H$_2$O$_2$ treatment (Figure 5c) because the actin filaments activity downstream of ABA-induced stomatal closure was damaged. Besides, this defect unexpectedly recovered marginally in response to a decline in ABA content and subsequently decreased in response to a further decrease in ABA content (Figure 7c,d). This revealed that the ABA content in ds8 could even enhance the severity of the stomatal closure deficiency when it exceeds a certain content. All these results suggested that DS8 was indispensable for ABA-induced stomatal closure. When DS8 was invalid, the impaired actin filaments activity interfered with the ABA-mediated stomatal closure.

Although the SCAR/WAVE complex and Arp2/3 complex were reported to work together to regulate actin filaments modeling (Pollard, 2007; Yanagisawa et al., 2015), there are still some doubts about their functions. In Arabidopsis, mutants of Arp2/3 complex subunits show reduced sensitivity to ABA-regulated stomatal closure (Jiang et al., 2012; Li et al., 2014). However, the mutant of PIR, a subunit of the SCAR/WAVE complex, shows normal sensitivity to ABA-regulated stomatal closure. This contradiction leaves two explanations. Firstly, the residual SCAR/WAVE complex activity in the PIR mutant is sufficient for the activation of Arp2/3 complex, so the activity of actin filaments is not affected. Secondly, the SCAR/WAVE complex is not involved in ABA-mediated stomatal closure (Isner et al., 2017). In our study, the insensitivity of ds8 to ABA treatment (Figure 5c) prompted us to support the former explanation.
Drought sensitive 8 affects drought sensitivity

The dysfunction of DS8 resulted in abnormal development of the leaf epidermis, including impaired cuticles and increased stomatal density. Moreover, the impaired actin cytoskeleton in ds8 led to defective ABA-mediated stomatal closure. All of these defects in leaf traits rendered rice plants suffering excessive water loss and increased sensitivity to drought. The impaired actin filaments in ds8 leaves promoted the accumulation of endogenous ABA, leading to accelerated leaf senescence. In summary, DS8 affects the sensitivity of rice plants to drought stress. Our findings reveal a genetic and molecular mechanism for the involvement of DS8 in the response of rice to drought stress.

EXPERIMENTAL PROCEDURES

Plant materials and culture conditions

Plants were grown in a paddy field at the China National Rice Research Institute in Hangzhou under normal irrigation throughout the growth period (5-8 cm shallow water depth during early vegetative stage; saturated soil condition during heading stage and early filling stage). For ABA content measurement, leaves of wild-type and mutant plants were harvested at the seedling stage, heading stage and 15 days after flowering. Flag leaves of wild-type and mutant plants were collected during the heading stage and filling stage for specific analysis.

Morphological observation of actin filaments

Alexa Fluor 488-phalloidin staining of actin filaments was performed as described previously (Olyslaegers and Verbeelen, 1998). To observe actin filaments, root tips were excised from seedlings and washed twice with PEM buffer (0.1 M PIPES, 10 mM EGTA, 0.3 M mannitol, 5 mM MgSO4, pH 6.9). The tissues were fixed in 4% paraformaldehyde in PEM buffer for 1 h, and washed twice with PEM buffer. The samples were stained with 0.66 mM Alexa Fluor 488-phalloidin (Life Technologies, Eugene, OR, USA) in PEM buffer for 1 h and visualized under a confocal laser-scanning microscope (LSM 700, Zeiss, Oberkochen, Germany).

Observation of cuticular layer and quantification of wax and cell wall components

For TEM observations of the cuticular layer, the middle parts of flag leaves were harvested during the heading stage, fixed in pre-cooled fixation buffer (0.1 M phosphate buffer containing 2.5% glutaraldehyde, pH 7.0) for at least 8 h, washed using phosphate buffer (0.1 M, pH 7.0) and dehydrated using a graded ethanol series. The tissue samples were then transferred to acetone for 20 min, embedded with resin, sectioned, stained with uranyl acetate and lead citrate, and visualized by TEM (H-7650, Hitachi, Tokyo, Japan). For wax components quantification, flag leaves were harvested during the heading stage. Wax components were quantified by the Metabolomics Facility of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. The test was repeated four times, and flag leaves collected from six plants were used each time. The content of cell wall components was analyzed according to Zhong and Lauchli (1993). The test was repeated three times, and flag leaves collected from six plants were used each time.

Scanning electron microscopy and paraffin section analysis

For scanning electron microscopy (SEM) analysis of the upper leaf epidermis, the middle parts of flag leaves were harvested during the heading stage, fixed in pre-cooled fixation buffer (0.1 M phosphate buffer containing 2.5% glutaraldehyde, pH 7.0) for at least 8 h, washed using phosphate buffer (0.1 M, pH 7.0), and dehydrated using a graded ethanol series. The tissue samples were then transferred to isoamyl acetate, dried, coated with gold and visualized by SEM (Hitachi TM-1000). For paraffin
section analysis, the middle parts of flag leaves were collected from wild-type and mutant plants, and fixed in 50% FAA (0.9 M glacial acetic acid, 3.7% formaldehyde and 50% ethanol) overnight at 4°C. The paraffin section analysis was performed according to Ren et al. (2016).

**Analysis of stomatal aperture status and abscisic acid treatment**

To analyze the stomatal aperture status of wild-type and mutant leaves, the seedlings cultured under field conditions were used. The middle part of the upper leaf epidermis was covered with transparent nail polish and allowed to dry for about 5 min. The nail polish cast, which could provide an impression of the leaf surface, was removed from the leaf and attached to a microscope slide with transparent adhesive tape (Peel et al., 2017). The stomatal apertures status was observed under a 90° light microscope (Nikon, Tokyo, Japan) at 10 x 40 amplification. Stomatal types were defined and analyzed according to Matsuda et al. (2016).

For ABA and H$_2$O$_2$ treatment, germinated seeds were sown in reconstructive 96-well plates and cultivated in distilled water for 3 days (12 h light at 30°C and 12 h dark at 25°C, 70% humidity). The seedlings were transferred to 1/2 Kimura B nutrient solution (Ueno et al., 2009) and cultivated for 10 days without changing the environmental conditions. Leaves were excised from 13-day-old seedlings and incubated in MES buffer (30 mM KCl, 0.1 mM CaCl$_2$, 10 mM MES-Tris, pH 6.1) for 90 min in the light (400 μmol m$^{-2}$ s$^{-1}$), photon flux density and 500 μmol m$^{-2}$ s$^{-1}$ air flow rate. The chlorophyll content was determined by spectrophotometry (Ye et al., 2016). The middle parts of flag leaves were collected from wild-type and mutant plants cut into segments of about 1 cm each. The leaf samples were immersed in 80% acetone for 24 h (26°C in dark) and measured by DU800 ultraviolet spectrophotometer (Beckman, Fullerton, CA, USA).

**Sodium tungstate and cytochalasin B treatment**

For sodium tungstate treatment, germinated seeds were sown in reconstructive 96-well plates and placed in an incubator (12 h light at 30°C and 12 h dark at 25°C, 70% humidity), cultivated in distilled water for 3 days and in 1/2 Kimura B nutrient solution for 10 days. Then, the 13-day-old mutant seedlings were transferred to 1/2 Kimura B nutrient solution containing 0, 0.5, 1.0, 1.5 or 2.0 mM sodium tungstate for 7 days. Thirteen-day-old wild-type seedlings were transferred to 1/2 Kimura B nutrient solution containing 0 mM sodium tungstate as a control. For CB treatment, 13-day-old wild-type seedlings were transferred to 1/2 Kimura B nutrient solution containing 0.05% DMSO or 5 μM CB (dissolved in 0.05% DMSO). The ABA content and stomatal aperture status were analyzed according to the before-mentioned methods in ‘Measurement of ABA, ROS content and electrolyte leakage’ and ‘Analysis of stomatal aperture status and ABA treatment’.

**Construct generation and transformation**

For the complementation test, a 14.8-kb genomic fragment containing the entire ORF of LOC_Os08g43130 and its native promoter were amplified from NPB genomic DNA and introduced into binary vector pCAMBIA1300 to generate the pDSS::DS8 plasmid. The plasmid was used to transform ds8 calli by Agrobacterium-mediated transformation. A GUS reporter construct containing the native DSS promoter was introduced into wild-type rice plants to examine the expression pattern of DSS. To determine the subcellular localization of DSS, a p35S::DSS-eGFP construct containing the full-length DSS CDS without a stop codon was generated. Both the empty p35S::eGFP vector (as a control) and the p35S::DSS-eGFP construct were introduced into wild-type rice protoplasts and calli. A DSS-antisense construct containing the reverse DSS CDS fragment under the control of the 35S promoter was generated and transformed into the wild-type to produce DSS antisense transgenic lines. The primer sequences used are listed in Table S3.

**Accession numbers**

Sequence data from this article can be found in the NCBI website (http://www.ncbi.nlm.nih.gov) under the following accession numbers: Os06g0130900 (Histone H3), Os08g0544500 (DSS).

**ACKNOWLEDGEMENTS**

The authors thank Guosheng Xiong (Nanjing Agricultural University) for critical reading and suggestions for our manuscript. This work was supported by the National Natural Science Foundation of China (grant no. 31661143006, 91735304, 91735303), the Ministry of Agriculture of China for transgenic research (grant no. 2016ZX08009003-001), and the Science and Technology Innovation Project of the Chinese Academy of Agriculture Sciences.

**CONFLICT OF INTEREST**

The authors declare no conflict of interests.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article.

**Figure S1.** Dysfunction of DSS increases the negative effects of a dry environment on rice production.

**Figure S2.** Phenotypes of various rice lines.
Figure S3. Expression pattern of DS8 and subcellular localization of DS8.

Figure S4. DS8 encodes a putative NAP1-like protein.

Figure S5. Protein sequence alignment of DS8 and its homologs from several species.

Figure S6. Analysis of cell wall components and monosaccharide content.

Figure S7. ds8 exhibits withered leaf tips and reduced chlorophyll content.

Table S1. Phenotypic data of wild-type and ds8 under normal and dry environmental conditions.

Table S2. Reciprocal crosses between ds8 and wild-type indica cultivars.

Table S3. Primers used in this study.

Table S4. Water loss rates of excised leaves harvested from transgenic complementation lines at the heading stage.

Table S5. Water loss rates of excised leaves harvested from WT, ds8 and antisense transgenic plants at the heading stage.

REFERENCES

Agarie, S., Uchida, H., Agata, W., Kubota, F. and Kaufman, P.B. (1998) Effects of silicon on transpiration and leaf conductance in rice (Oryza sativa L.). Plant Prod. Sci. 1, 89–95.

Bai, J.T., Zhu, X.D., Wang, Q. et al. (2016) Rice TUTOU1 encodes a suppressor of CAM3 receptor-like protein that is important for actin organization and panicle development. Plant Physiol. 169, 1179–1191.

Basu, D., Seld, E.A., Le, J., Mallery, E.L. and Szymanski, D.B. (2005) NAP1 is essential for Arp2/3-dependent nuclearation and assembly of actin filaments controls cell elongation in Arabidopsis. Plant Physiol. 137, 2335–2340.

Buckley, T.N. (2005) The control of stomata by water balance. New Phytol. 168, 275–292.

Cao, L.Y., Henty-Ridilla, J.L., Blanchoin, L. and Staiger, C.J. (2016) Profilin-dependent nucleation and assembly of actin filaments controls cell elongation in Arabidopsis. Plant Physiol. 170, 220–233.

Chen, Z.H., Chen, G., Dai, F., Wang, Y.Z., Hills, A., Ruan, Y.L., Zhang, G.P., Franks, P.J., Nevo, E. and Blatt, M.R. (2017) Molecular Evolution of Grass Stomata. Trends Plant Sci. 22, 124–139.

Deeks, M.J., Kaloriti, D., Davies, B., Malhi, A., Samanta, M.K., Gayen, S. and Maiti, M.K. (2016) A small, novel protein highly conserved in plants and animals promotes the polarized growth and division of maize leaf epidermal cells. Curr. Biol. 26, 849–853.

Gan, L., Wang, X., Cheng, Z. et al. (2016) Wax crystal-sparsé leaf 3 encoding a β-ketocacyl-CoA reductase is involved in cuticular wax biosynthesis in rice. Plant Cell Rep. 35, 1687–1698.

Gao, X., Zou, C., Wang, L. and Zhang, F. (2005) Silicon improves water use efficiency in maize plants. J. Plant Nutr. 27, 1457–1470.

Gao, X.Q., Wang, X.L., Ren, F., Chen, J. and Wang, X.C. (2009) Dynamics of vacuoles and actin filaments in guard cells and their roles in stomatal movement. Plant Cell Environ. 32, 1108–1116.

Gao, Y., Wu, M.Q., Zhang, M.J. et al. (2018) A maize phytochrome-interacting factors protein ZmPIF1 enhances drought tolerance by inducing stomatal closure and improves grain yield in Oryza sativa. Plant Biotechnol. J. 16, 1375–1387.

Geiger, D., Scherzer, S., Mumm, P. et al. (2010) Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca2+ affinities. Proc. Natl Acad. Sci. USA 107, 8023–8028.

Gudesblat, G.E., Iusem, N.D. and Morris, P.C. (2007) Guard cell-specific inhibition of Arabidopsis MPK3 expression causes abnormal stomatal responses to abscisic acid and hydrogen peroxide. New Phytol. 173, 713–721.

Guo, J.J., Yang, X.H., Weston, D.J. and Chen, J.G. (2011) Abscisic acid receptors, past, present and future. J. Integr. Plant Biol. 53, 469–479.

Hardham, A.R., Jones, D.A. and Takekoto, D. (2007) Cytoskeleton and cell wall function in penetration resistance. Curr. Opin. Plant Biol. 10, 342–348.

Hayaki, T., Kutsuna, N., Sano, T., Kondo, N. and Hasezawa, S. (2010) Quantification and cluster analysis of actin cytoskeletal structures in plant cells, role of actin bundling in stomatal movement during diurnal cycles in Arabidopsis guard cells. Plant J. 61, 156–165.

Hu, Y., Wu, Q., Peng, Z. et al. (2011) Silencing of OsGRXS17 in rice improves drought stress tolerance by modulating ROS accumulation and stomatal closure. Sci. Rep. 1, 15 950.

Huang, X.Y., Chao, D.Y., Gao, J.P., Zuo, M.Z., Shi, M. and Lin, H.X. (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Gene Dev. 23, 1805–1817.

Jafar, J.C., Xu, Z., Costa, J.M., Monnet, F., Batsone, T., Ou, X., Deeks, M.J., Genty, B., Jiang, K. and Hetherington, A.M. (2017) Actin filament reorganisation controlled by the SCAR/WAVE complex mediate stomatal response to darkness. New Phytol. 215, 1059–1067.

Jiang, K., Sorefan, K., Deeks, M.J., Bevan, M.W., Hussey, P.J. and Hetherington, A.M. (2012) The ARP2/3 complex mediates guard cell actin reorganisation and stomatal movement in Arabidopsis. Plant Cell 24, 2031–2040.

Jin, Z.P., Xue, S.W., Luo, Y.A., Tian, B.H., Fang, H.H., Li, H. and Pei, Y. (2013) Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in Arabidopsis. Plant Physiol. Biochem. 62, 41–46.

Kang, J., Huang, J.U., Lee, M., Kim, Y.Y., Assmann, S.M., Martinoia, E. and Lee, Y. (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl Acad. Sci. USA 107, 2355–2360.

Kim, T.H., Bohmer, M., Hu, H., Nishimura, N. and Schroeder, J.I. (2010) Guard cell signal transduction network, advances in understanding abscisic acid, CO2, and Ca2+ signaling. Annu. Rev. Plant Biol. 61, 561–591.

Kunst, L. and Samuels, A.L. (2003) Biosynthesis and secretion of plant cuticular wax. Prog. Lipid Res. 42, 51–80.

Le, J., Mallory, E.L., Zhang, C.H., Brankle, S. and Szymanski, D.B. (2006) Arabidopsis BRICK1/HSFPC300 is an essential WAVE-complex subunit that selectively stabilizes the Arp2/3 activator SCAR2. Curr. Biol. 16, 985–991.

Lemichez, E., Wu, Y., Sanchez, J.P., Mettouhchi, A., Mauthur, J. and Chua, N.H. (2001) Inactivation of ATR1 by abscisic acid is essential for stomatal closure. Gene Dev. 15, 1808–1816.

Li, X., Li, J.H., Wang, W., Chen, N.Z., Ma, T.S., Xi, Y.N., Xiao, L.Z., Hai, F.L., Bai, Y. and Huang, S.J. (2014) ARP2/3 complex-mediated actin dynamics is required for hydrogen peroxide-induced stomatal closure in Arabidopsis. Plant Cell Environ. 37, 1548–1560.

Fang, Y. and Xiong, L. (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci. 72, 873–889.

Frank, M.J. and Smith, L.G. (2002) A small, novel protein highly conserved in plants and animals promotes the polarized growth and division of maize leaf epidermal cells. Curr. Biol. 12, 849–853.

Gao, X., Zou, C., Wang, L. and Zhang, F. (2005) Silicon improves water use efficiency in maize plants. J. Plant Nutr. 27, 1457–1470.
Li, X., Han, H., Chen, M., Yang, W., Liu, L., Li, N., Ding, X.H. and Chu, Z.H. (2016) Overexpression of OsDT1, which encodes a novel cysteine-rich peptide, enhances drought tolerance and increases ABA concentration in rice. Plant Mol. Biol. 93, 21-34.

Liu, J.P., Zhang, C.G., Wei, C.C., Liu, X., Wang, M.G., Yu, F.F., Xie, Q. and Tu, J.M. (2016) The RING finger ubiquitin E3 ligase OsTAS enhances heat tolerance by promoting H2O2-induced stomatal closure in rice. Plant Physiol. 170, 429-443.

Ma, J.F. and Yamaji, N. (2008) Functions and transport of silicon in plants. Cell. Mol. Life Sci. 65, 3049-3057.

Machesky, L.M., Mullins, R.D., Higgs, H.N., Kaiser, D.A., Blanchon, L., May, R.C., Hall, M.E. and Pollard, T.D. (1999) Scar, a WASP-related protein, activates nucleation of actin filaments by the Arp2/3 complex. Proc. Natl. Acad. Sci. USA 96, 3739-3744.

MacRobbie, E.A. and Kurup, S. (2007) Signaling mechanisms in the regulation of vacuolar ion release in guard cells. New Phytol. 175, 630-640.

Mao, B.G., Cheng, Z.J., Lei, C.L. et al. (2016) Rice stomatal closure requires guard cell plasma membrane ATP-binding cassette transporter RCH1/OsABCG5. Mol. Plant 9, 417-427.

McCurdy, D.W., Kovar, D.R. and Staiger, C.J. (2001) Actin and actin-binding proteins in higher plants. Protoplasma 215, 89-104.

Mendoza, M.C. (2013) Phosphoregulation of the WAVE regulatory complex and signal integration. Semin. Cell Dev. Biol. 24, 272-279.

Miao, C.B., Xiao, L.H., Hua, K., Zou, C.S., Zhao, Y., Bressan, R.A. and Zhu, J.K. (2018) Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. Proc. Natl Acad. Sci. USA 115, 6098-6103.

Müller, R. and Blumwald, E. (2015) The roles of ROS and ABA in systemic acquired acclimation. Plant Cell 27, 64-70.

Moradi, F. and Ismael, A.M. (2007) Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seeding and reproductive stages in rice. Ann. Bot. London 99, 1161-1173.

Morita, R., Sato, Y., Masuda, Y., Nishimura, M. and Kusaba, M. (2009) Defect in non-yellow coloring 3, an Arabidopsis GNARLED homolog that regulates chloroplast development and abiotic stress response in rice. Proc. Natl Acad. Sci. USA 106, 4063.

Muthukrishnan, K. and Fluhr, R. (1962) Histochemistry of silicon in leaves of Abnormal maize. Protoplasma 48, 630-632.

Nakanishi, S., Ohto, H., Ishimaru, T., Maeshima, K., Myouga, F., Toyooka, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2013) SNAC-As, stress-responsive NAC transcription factors, mediate ABA-inducible leaf senescence. Plant J. 84, 1114-1123.

Planta 235, 39-52.

Ren, D.Y., Rao, Y.C., Wu, L.W. et al. (2016) The pleiotropic ABNORMAL FLOWER AND DWARF1 affects plant height, floral development and grain yield in rice. J. Integr. Plant Biol. 58, 529-539.

Rice. Plant Physiol. 171, 1569-1590.

Sussmilch, F.C. and McAdam, S.A. (2017) Surviving a dry future, abscisic acid (ABA)-mediated plant mechanisms for conserving water under low humidity. Plants 6, 54.

Takasaki, H., Maruyama, K., Takahashi, F., Fujita, M., Yoshida, T., Nakashima, K., Myouga, F., Toyooka, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2015) SNAC-As, stress-responsive NAC transcription factors, mediate ABA-inducible leaf senescence. Plant J. 84, 1114-1123.

Thomas, C. and Staiger, C.J. (2014) A dynamic interplay between membranes and the cytoskeleton critical for cell development and signaling. Front. Plant Sci. 5, 335.

Unno, D., Koyama, E., Kono, I., Ando, T., Yano, M. and Ma, J.F. (2009) Identification of a novel major quantitative trait locus controlling distribution of C3 between roots and shoots in rice. Plant Cell Physiol. 50, 2223-2233.

Wang, X., Guan, Y., Zhang, D., Dong, X., Tian, L. and Qu, L.Q. (2017) A \( \beta \)-ketocycl-CoA synthase is involved in rice leaf cuticular wax synthesis and requires a CER2-LIKE protein as a cofactor. Plant Physiol. 173, 944-955.

Welch, M.D. and Mullins, R.D. (2002) Cellular control of actin nucleation. Annu. Rev. Cell Dev. Biol. 18, 247-288.

Wu, R., Li, S., He, S., Wassmann, F., Yu, C., Qin, G., Schreiber, L., Ou, J.J. and Gu, H. (2011) CFl1, a WW domain protein, regulates cuticle development by modulating the function of HDG1, a class IV homodomain transcription factor, in rice and Arabidopsis. Plant Cell 23, 3392-3411.

Wu, L.W., Ren, D.Y., Hu, S.K. et al. (2016) Down-regulation of a nicotinate phosphoribosyltransferase gene, OsNaPT1, leads to withered leaf tips. Plant Physiol. 171, 1085-1089.

Yamagawa, M., Desyatova, A.S., Belteton, S.A., Mallery, E.L., Turner, J.A. and Szymanski, D.B. (2015) Patterning mechanisms of cytoskeletal and cell wall systems during leaf trichome morphogenesis. Nat. Plants 1, 1504.

Ye, W.J., Hu, S.K., Wu, L.W. et al. (2016) White stripe leaf 12 (WSL12), encoding a nicotinamide diphosphate kinase 2 (OsNDPK2), regulates chloroplast development and abiotic stress response in rice (Oryza sativa L.). Mol. Breed. 36, 57.

Yoo, C.Y., Pence, H.E., Jin, J.B., Miura, K., Gosney, M.J., Hasegawa, P.M. and Mickelbart, M.V. (2010) The Arabidopsis GTL1 transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via transpression of SDD1. Plant Cell 22, 4128-4141.

Yoshida, S., Ohsahi, T. and Kitagishi, K. (1992) Histochemistry of silicon in rice plant, III. The presence of cuticle-silica double layer in the epidermal tissue. Soil Sci. Plant Nutr. 8, 1-5.

Yu, D., Ranathunge, K., Huang, H., Pei, Z., Franke, R., Schreiber, L. and He, C. (2008) Wax Crystal-Sparse Leaf1 encodes a \( \beta \)-ketocycl-CoA synthase involved in biosynthesis of cuticular waxes on rice leaf. Planta 228, 675-685.

Zhang, X., Zhang, L., Dong, F., Gao, J., Galbraith, D.W. and Song, C.P. (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in Vicia faba. Plant Physiol. 126, 1438-1448.

Zhang, W., Fan, L.M. and Wu, W.H. (2007) Osmo-sensitive and stretch-activated calcium-permeable channels in Vicia faba guard cells are regulated by actin dynamics. Plant Physiol. 142, 1140-1151.

Zhao, Y., Zhao, S.S., Mao, T.L. et al. (2011) The plant-specific actin binding protein SCAB1 stabilizes actin filaments and regulates stomatal movement in Arabidopsis. Plant Cell 23, 2314-2330.

Zhao, Y., Chan, Z., Gao, J. et al. (2016) ABA receptor PYL9 promotes drought resistance and leaf senescence. Proc. Natl Acad. Sci. USA 113, 1849-1854.

© 2019 The Authors.

The Plant Journal published by John Wiley & Sons Ltd and Society for Experimental Biology., The Plant Journal, (2019), 98, 884–897.
Zhong, H. and Lauchli, A. (1993) Changes of cell wall composition and polymer size in primary roots of cotton seedlings under high salinity. J. Exp. Bot. 44, 773–778.

Zhou, W.Q., Wang, Y.H., Wu, Z.L., Liang, L., Ping, L., Yan, L.F. and Hou, S.W. (2016) Homologs of SCAR/WAVE complex components are required for epidermal cell morphogenesis in rice. J. Exp. Bot. 67, 4311–4323.

Zhu, X. and Xiong, L. (2013) Putative megaenzyme DWA1 plays essential roles in drought resistance by regulating stress-induced wax deposition in rice. Proc. Natl Acad. Sci. USA 110, 17790–17795.