Journal of Experimental Botany, Vol. 69, No. 9 pp. 2403–2414, 2018
doi:10.1093/jxb/ery089 Advance Access publication 10 March 2018

RESEARCH PAPER

The interaction of strigolactones with abscisic acid during the drought response in rice

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Received 3 November 2017; Editorial decision 27 February 2018; Accepted 8 March 2018

Editor: Richard Napier, University of Warwick, UK

Abstract

Both strigolactones (SLs) and abscisic acid (ABA) biosynthetically originate from carotenoids. Considering their common origin, the interaction of these two hormones at the biosynthetic and/or regulatory level may be anticipated. Here we show that, in rice, drought simultaneously induces SL production in the root, and ABA production and the expression of SL biosynthetic genes in the shoot. Under control conditions, the ABA concentration was higher in shoots of the SL biosynthetic rice mutants *dwarf10* (*d10*) and *d17* than in wild-type plants, while a similar trend was observed for the SL perception mutant *d3.* These differences were enhanced under drought. However, drought did not result in an increase in leaf ABA content in the rice mutant line *d27*, carrying a mutation in the gene encoding the first committed enzyme in SL biosynthesis, to the same extent as in the other SL mutants and the wild type. Accordingly, *d10*, *d17*, and *d3* lines were more drought tolerant than wild-type plants, whereas *d27* displayed decreased tolerance. Finally, overexpression of *OsD27* in rice resulted in increased levels of ABA when compared with wild-type plants. We conclude that the SL and ABA pathways are connected with each other through D27, which plays a crucial role in determining ABA and SL content in rice.

Keywords: Abscisic acid, biosynthesis, drought, DWARF27, rice, strigolactones.

Introduction

Due to their sessile nature, plants must adjust their growth and development to continuously changing environmental conditions (Wolters and Jürgens, 2009). Plant hormones, such as abscisic acid (ABA), auxin, cytokinin, gibberellin, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and strigolactones (SLs), play a central role in regulating these adaptive

Abbreviations: ABA, abscisic acid; CCD, carotenoid cleavage dioxygenase; D27, DWARF27; FC, field capacity; SL, strigolactone.

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responses (Santner and Estelle, 2009). However, plant hormones do not act alone but are interconnected and modulate each other's activity at the level of biosynthesis, degradation, and signalling by crosstalk mechanisms (Zhang *et al.*, 2013).

ABA is a well-studied phytohormone that not only regulates several developmental processes such as seed maturation and dormancy, but is also intricately involved in the adaptation of plants to abiotic stresses (i.e. drought, salinity, etc.) (Nambara and Marion-Poll, 2005; Finkelstein, 2013). It is generally accepted that under drought, the ABA level accumulates in the root and is then transported to the shoot via the xylem, which results in stomatal closure that limits water loss and the accumulation of reactive oxygen species (ROS) (Ko and Helariutta, 2017). However, it has been shown that ABA is also directly generated in the shoot in response to drought (Borghi *et al.*, 2015). In addition, ABA stimulates the formation of DEHYDRINS and LATE EMBRYOGENESIS ABUNDANT (LEA) proteins that are involved in osmotic adjustment and other protection mechanisms (Shinozaki and Yamaguchi-Shinozaki, 2007). In plants, ABA is derived from C_{40}-carotenoid precursors synthesized in the plastids (Moise *et al.*, 2014; Nisar *et al.*, 2015). The first committed step in ABA biosynthesis is the oxidative cleavage of the epoxy-carotenoids 9-*cis*-violaxanthin or 9'-*cis*-neoxanthin to xanthoxin (Schwartz *et al.*, 1997), catalysed by 9-*cis*-epoxycarotenoid dioxygenases (NCEDs) and considered to be the rate-limiting step in ABA biosynthesis (Tan *et al.*, 1997; Burbidge *et al.*, 1999; Schwartz *et al.*, 2003). Xanthoxin is further converted, in the cytosol, by a short-chain dehydrogenase/reductase (SDR) to abscisic aldehyde that is oxidized to form ABA by abscisic aldehyde oxidase (AAO3) (Seo *et al.*, 2000; Cheng *et al.*, 2002) (Supplementary Fig. S1 at *JXB* online).

SLs were initially isolated from root exudates as seed germination stimulants of root parasitic plants of the Orobanchaceae (*Striga*, *Phelipanche*, and *Orobanche* spp.) (Cook *et al.*, 1966; Bouwmeester *et al.*, 2003; Xie *et al.*, 2010) and later identified as hyphal branching stimulants of arbuscular mycorrhizal fungi (AMF) (Akiyama *et al.*, 2005). Some years later, SLs were also recognized as hormones involved in the inhibition of shoot branching (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008) and a range of other biological processes, including root development, hypocotyl growth, responses to environmental conditions, photo-morphogenesis, and secondary growth in vascular plants, as well as protonema branching and colony extension in the moss *Physcomitrella patens* (Ruyter-Spira *et al.*, 2013; Zhang *et al.*, 2013; Al-Babili and Bouwmeester, 2015). When growing under low phosphate levels, plants display increased biosynthesis and exudation of SLs (Yoneyama *et al.*, 2007), which stimulates the hyphal branching of symbiotic AMF that acquire phosphate from the soil and provide it to the host in return for photoassimilates (Bouwmeester *et al.*, 2007; López-Ráez *et al.*, 2008).

Based on studies with mutants, so far eight genes have been identified in several plant species, with roles in SL biosynthesis and signalling (Waters *et al.*, 2017; Jia *et al.*, 2018). Among them, five have been shown to be required for SL biosynthesis. In rice and Arabidopsis, *DWARF27* (*D27*) (Lin *et al.*, 2009; Waters *et al.*, 2012) encodes a β-carotene isomerase converting all-*trans*-β-carotene into 9-*cis*-β-carotene (Alder *et al.*, 2012). *MORE AXILLARY GROWTH 3* (*MAX3*)/*DWARF17* (*D17*) (Booker *et al.*, 2004; Zou *et al.*, 2006) and *MORE AXILLARY GROWTH 4* (*MAX4*)/*DWARF10* (*D10*) (Sorefan *et al.*, 2003; Arite *et al.*, 2007) encode the carotenoid cleavage dioxygenases (CCDs), CCD7 and CCD8, respectively. *MORE AXILLARY GROWTH 1* (*MAX1*) encodes a cytochrome P450 monooxygenase (Booker *et al.*, 2005). *In vitro* studies with D27, CCD7, and CCD8 showed that these enzymes are sufficient to convert all-*trans*-β-carotene into carlactone (CL), an intermediate with obvious structural similarities to canonical SLs (Alder *et al.*, 2012; Seto *et al.*, 2014). The formation of CL starts with the D27-catalysed isomerization of all-*trans*-β-carotene (Bruno and Al-Babili, 2016), followed by the CCD7-catalysed stereospecific cleavage (Bruno *et al.*, 2014) that leads to the intermediate 9-*cis*-β-apo-10'-carotenal that is then converted by CCD8 into CL (Alder *et al.*, 2012; Bruno *et al.*, 2017). Recently, we demonstrated that a rice MAX1 homologue (carlactone oxidase) catalyses the conversion of CL into the SL parent molecule, 4-deoxyorobanchol (*ent*-2′-*epi*-5-deoxystrigol), by forming the B–C lactone ring, and that a second rice MAX1 homologue (orobanchol synthase) forms orobanchol by introducing a hydroxyl group into 4-deoxyorobanchol (Zhang *et al.*, 2014). Arabidopsis MAX1 has been shown to oxidize CL to a different SL-like molecule, carlactonic acid, which is methylated into methyl-carlactonoate (MeCLA)—that can bind to the receptor D14 (see below)—by an as yet unidentified enzyme (Abe *et al.*, 2014). More recently, Brewer *et al.* (2016) revealed that in Arabidopsis LATERAL BRANCHING OXIDOREDUCTASE (LBO), an oxidoreductase-like enzyme of the 2-oxoglutarate and Fe(II)-dependent dioxygenase (2OGD) superfamily, converts MeCLA into an unidentified SL-like compound (Brewer *et al.*, 2016). SL mutants have a high-tillering/branching phenotype, which in the case of biosynthetic mutants can be rescued by exogenous application of GR24 (Wang and Li, 2011), but not in the SL perception and downstream signalling mutants (Umehara *et al.*, 2008). Up to now, there are three genes known to be involved in the latter process: *MORE AXILLARY GROWTH 2* (*MAX2*)/*DWARF3* (*D3*) encodes an F-box protein (Stirnberg *et al.*, 2007); *DWARF14* (*D14*), an α/β-fold hydrolase (Hamiaux *et al.*, 2012, Nakamura *et al.*, 2013; de Saint Germain *et al.*, 2016; Yao *et al.*, 2016); and *SUPPRESSOR OF MORE AXILLARY GROWTH2-LIKE 6,7,8* (*SMXL6,7,8*)/*DWARF53* (*D53*) that encode repressors of SL signalling (Jiang *et al.*, 2013; Zhou *et al.*, 2013; Wang *et al.*, 2015; Soundappan *et al.*, 2015).

Considering their common biosynthetic origin from carotenoids (Matusova *et al.*, 2005), a relationship between ABA and SL production may be anticipated. Indeed, ABA-deficient tomato mutants impaired in different steps of ABA biosynthesis exhibit reduced SL levels (López-Ráez *et al.*, 2008, 2010), suggesting that ABA affects SL accumulation. Conversely, Torres-Vera *et al.*, (2014) reported that the SL-deficient tomato mutant *slccd8* had reduced levels of ABA. Similarly, an SL-deficient *CCD7*RNAi lotus line showed reduced ABA levels when exposed to a combination

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of phosphate deficiency and osmotic stress, compared with the corresponding wild type (Liu *et al.*, 2015).

Because in the above-mentioned studies SL deficiency was shown to reduce ABA levels, it can be expected that SL-deficient plants are compromised in their response to drought. Interestingly, two independent studies investigated drought tolerance in Arabidopsis SL mutants (Bu *et al.*, 2014; Ha *et al.*, 2014). Ha *et al.* (2014) reported that SLs act as a positive regulator in the plant's response to drought and salt stress. The exogenous application of GR24 rescued the drought-sensitive phenotype of SL-deficient mutants (*max3* and *max4*) but not of the insensitive mutant (*max2*). In addition to this, the *max* mutants showed reduced sensitivity to exogenous ABA compared with wild-type plants. In the study by Bu *et al.* (2014), the drought sensitivity phenotype was confined to *max2*. However, ABA levels in the wild type and *max2* were similar under control and drought conditions.

To obtain a better understanding of the relationship between SLs and ABA during drought, in the present study we first studied whether SLs are affected by drought. Hereto, we quantified SL content and analysed the expression level of SL biosynthesis genes in rice exposed to drought conditions. Furthermore, we investigated the consequences of mutations in SL biosynthesis and signalling and *D27* overexpression on ABA content. Our results show that SL biosynthesis/perception interferes with ABA formation as observed in *d10*, *d17*, and *d3* mutants, and suggest that the SL biosynthetic enzyme D27 is unique in its ability to regulate the levels of both plant hormones.

Materials and methods

Plant materials, growth conditions, and drought treatments

Rice (*Oryza sativa* L. cv. Shiokari) was used in this study. Rice seeds were first surface sterilized with 70% ethanol for 30 s and with 2% sodium hypochlorite (v/v) for 30 min. The seeds were then rinsed five times in sterile double-distilled water and immersed in water in the dark for 2 d at 28 °C to induce germination. Finally, germinated seeds were transferred to the climate room in pots filled with a mixture of sand and soil (1:1) (for the drought tolerance assay) or silver sand (for others). The conditions in the climate room were: temperature, 28 °C day/25 °C night; photoperiod, 10 h light/14 h dark; 75% relative humidity, and light intensity of 570 µM m^{-2} s^{-1}. The rice plants were watered twice a week using modified half-strength Hoagland nutrient solution (López-Ráez *et al.*, 2008).

To study the effect of long-term drought conditions in rice, four seedlings of *japonica* variety Shiokari were grown together in three litre volume pots. The pots were filled with silver sand equally (the same soil volume in all the pots) and watered at saturated levels until plants were 4 weeks old. After this stage, drought was imposed by water withholding until a field capacity (FC) of 45% was reached. This condition was maintained for 9 d. The drought was maintained by weighing the pots every day and watering with nutrient solution until the desired water level. After the drought period, plant material was collected for further biomass and hormone quantification. To study the effect of strong drought conditions, stress by water withholding was imposed on 2-week-old seedlings (five-leaf stage) during 10 d.

To test the drought tolerance of rice SL mutants (*d10*, *d17*, *d27*, and *d3*), seeds of mutants and the wild type were germinated on half-strength Murashige and Skoog (MS) medium. The rice seedlings (16 plants per pot and five pots for each line) were grown in a climate room in three litre pots (diameter 19 cm, depth 14.5 cm) filled with a mixture of sand and soil (1:1). To minimize experimental errors, each pot was filled with the same weight of soil and supplied with the same volume of water. The drought treatment and water loss rate experiment under dehydration conditions were performed according to Zhang *et al.* (2012) with minor modifications. When plants reached the five-leaf stage, watering was withheld for 12 d. Watering was then resumed for 3 d to allow plants to recover, after which the survival rates were calculated. To rule out the possible effect of differences in plant architecture between the rice lines studied for the drought resistant/sensitive phenotypes, a pooled survival experiment was also performed. In this experiment, eight plants from the *d27* line (drought sensitive) and eight plants from the *d10* line (drought resistant) were sown in the same pot with a total of eight replicates. The rest of the procedure was similar to the drought survival assay performed for the individual lines.

To detect the water loss rate under dehydration conditions, flag leaves were detached from plants (n=9) and weighed at 0, 0.5, 1, 2, 3, 4, 5, and 6 h after their removal from the plant (at 24 °C). Water loss rates were calculated as the percentage of initial fresh weight.

Generation of OsD27 *overexpression lines in rice*

Full-length cDNA of *OsD27* was PCR amplified using primers *D27-OE-F* and *D27-OE-R* (Supplementary Table S1), digested by *Bam*HI and *Spe*I, and then inserted into the corresponding sites downstream of the ubiquitin promoter in the binary vector pTCK303 (Wang *et al.*, 2004). The resulting plasmid was designated as *pUbi::D27* and introduced into rice variety Shiokari using *Agrobacterium tumefaciens* strain EHA105 as previously described (Hiei *et al.*, 1994).

Strigolactone and abscisic acid analysis

For SL analysis in rice, plants were grown, and root extracts were collected and extracted according to Jamil *et al.* (2011*b*). ABA was analysed as previously described (López-Ráez *et al.*, 2010) with minor modifications. For SL quantification from root extracts, 0.5 g of fresh root tissue was manually ground in liquid nitrogen. Samples were taken in 10 ml cold glass tubes and 2 ml of ethyl acetate containing GR24 [0.01 nmol ml^{-1} ethyl acetate solution of GR24 as internal standard (IS)] was added and mixed by vortexing. After 15 min sonication (in a Branson 3510 ultrasonic bath), each sample was centrifuged for 10 min at 2000 rpm. The organic phase was transferred to 4 ml glass vials. The samples were re-extracted with another 2 ml of ethyl acetate (without IS) and combined in the same 4 ml glass vials. The collected samples were dried in a speed vacuum (Thermo Scientific SPD121P SpeedVac). The residue was dissolved in 50 µl of ethyl acetate and diluted with 4 ml of 100% hexane. This solution was loaded in a pre-conditioned Silica gel GracePure SPE (200 mg/3 ml) column. After washing with 2 ml of 100% hexane, SL was eluted with 2 ml solvent mixtures of hexane:ethyl acetate (10:90). ABA was extracted using 0.2 g of leaf material in a 4 ml glass vial containing 1 ml of ethyl acetate and 0.01 D6-ABA (IS). After 15 min of sonication, each sample was centrifuged for 10 min at 2000 rpm. The organic phase was transferred to 4 ml glass vials. The samples were re-extracted with another 1 ml of ethyl acetate (without IS) and combined in the same 4 ml glass vials. The collected samples were dried in a speed vacuum and the residue was dissolved in 50 µl of methanol and diluted with 3 ml of water. This solution was loaded in a pre-conditioned C18 GracePure column (100 mg/1 ml). ABA was eluted with 1 ml of 100% acetone. The solvent mixtures were evaporated under speed vacuum and the residue was dissolved in 200 µl of acetonitrile:water (25:75, v/v). The samples were filtered through Minisart SRP4 0.45 µm filters (Sartorius, Germany) for ultra-performance liquid chromatography–tandem spectrometry (UPLC-MS/MS), as previously described (López-Ráez *et al.*, 2010; Jamil *et al.*, 2011*b*). Data acquisition and analysis were performed using MassLynx 4.1 (TargetLynx) software (Waters). Calculations of ABA and SL 4-DO concentration were based on a

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standard calibration curve and corrected by the IS. Calculation of the SL methoxy-5DS was based on peak area, since a standard was not available.

Analysis of transcript levels

Total RNA was extracted from rice roots and shoots using a combination of three protocols: TRIzol reagent (Invitrogent), the RNeasy Mini Kit (Qiagen), and the DNase-I Kit (Qiagen), as previously described (http://www.untergasser.de/lab/protocols/rna_prep_comb_trizol_v1_0.htm). RNA concentration, quality, and integrity were checked using a NanoDrop ND-1000 UV-Vis spectrophotometer and standard gel electrophoresis. Reverse transcription reaction was performed with the Bio-Rad iScript cDNA Synthesis Kit using 1 µg of total extracted RNA following the manufacturer's instructions. Primers used for real-time quantitative reverse transcription–PCR (qRT-PCR) analysis are listed in (Supplementary Table S1). qRT-PCR was performed with the Bio-Rad iQ5 instrument using Bio-Rad SYBR Green Supermix to monitor dsDNA synthesis following the manufacturer's instructions. Three independent biological replicates were used, and each PCR was done in triplicate. Relative expression levels of genes were determined using the comparative C_t method as previously described (Livak and Schmittgen, 2001), and the rice *Ubiquitin* (*ubi*) gene (Supplementary Table S1) was used as the internal control to normalize target gene expression (Lin *et al.*, 2009).

Stomatal density

Leaf stomatal density was determined by using the silicon rubber impression technique (Smith *et al.*, 1989). The method and impression material are described by Giday *et al.* (2013). Imprints of the leaf surface from the abaxial surfaces of the more developed leaf were taken from 6-week-old plants (three biological replicates). Imprints were later smeared with nail polish in the mid-area between the central vein and the leaf edge, and after drying the polish was peeled off from the imprint and mounted for microscope count. Numbers of stomata from three randomly selected non-overlapping rectangular fields of view (×20 magnification, 0.68 mm^2) per biological replicate were counted under a light microscope (Zeiss Imager D2, Germany) and Axiocam camera (Zeiss, Germany).

Carotenoid extraction from rice seedlings for quantitative HPLC analysis

The extraction was performed under dim light by adding 2 ml of acetone to 10 mg of finely ground powder of plant material, followed by sonication (duty-cycle 30, output 2) and centrifugation (5 min at 3000 *g*). This procedure was repeated three times, after which 2 ml of PE:DE (2:1, v/v) was added to the collected supernatants. Water was added up to 14 ml followed by centrifugation for 5 min at 3000 *g*. The organic phase was collected and the extraction was repeated once more. The combined organic phases were evaporated to dryness and dissolved in exactly 100 µl of $CHCl_3$—from which 10 µl was injected for HPLC analysis.

Substrates were quantified spectrophotometrically at their individual λ_{max} using molar absorption coefficients given in the literature (Britton *et al.*, 1995). Protein concentration was determined using the Bio-Rad protein assay kit. For HPLC analysis, a Waters system equipped with a photodiode array detector (model 996) was used. Separation was performed using YMC-Pack C_{30} reversed phase columns from YMC Europe (150 mm length×3 mm internal diameter, 5 µm particles, system 1; and 250 mm length×4.6 mm internal diameter, 5 µm particles, system 2). The column temperature was set at 35 °C. The solvent system consisted of solvent A, methanol/tert-butyl methyl ether (1:1, v/v); and B, methanol/tert-butyl methyl ether/water (5:1:1, v/v/v). In plant tissue analysis, the column was developed at a flow rate of 1 ml min^{-1} with 100% B followed by a gradient to 18% B within 65 min. Then, the flow was subsequently increased to 1.5 ml min^{-1} to 0% B within 2 min and maintained for another 11 min. Finally, the column was re-equilibrated to starting conditions for 10 min.

Results

SL production is induced under drought

To study the effect of drought on SL production, five-leaf stage rice plants were grown under a continuous, mild water deficit. Mild drought conditions resulted in a reduction in plant biomass, particularly of the shoot (Supplementary Fig. S2A), showing that this level of water deficit is indeed limiting plant growth. ABA increased in the shoot of mildly drought-stressed plants, but not in the roots (Supplementary Fig. S2B). SL levels strongly increased in the roots when plants were exposed to mild drought (Fig. 1). Roots of plants grown under drought contained a higher level of 4-deoxyorobanchol and a higher sum of the three putative methoxy-5-deoxystrigol isomers 1–3 (2-fold), identified before in rice (Jamil *et al.*, 2011*a*; Cardoso *et al.*, 2014), when compared with plants grown under control conditions (Fig. 1).

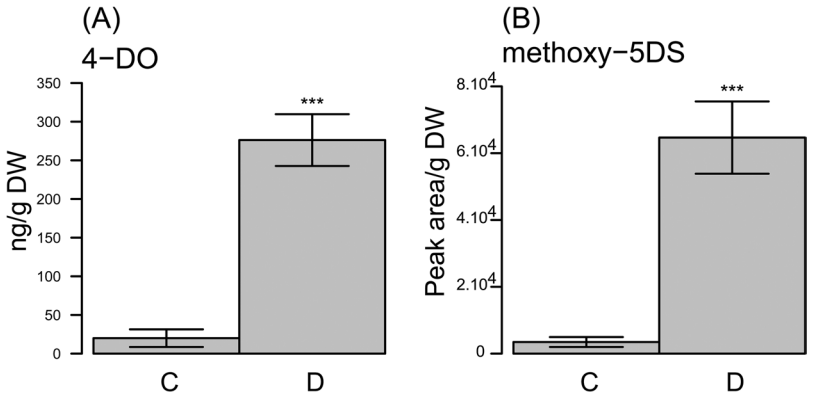

Fig. 1. Effect of mild drought on SL content in rice root extracts. Levels of 4-deoxyorobanchol (4-DO; A) and methoxy-5DS (B) (as determined by the sum of three methoxy-5-deoxystrigol isomers) were measured in root extracts of wild-type Shiokari. Roots were collected from 6-week-old rice plants (Experiment 1). Long-term mild drought (35–45% field capacity) was applied during 9 d. Bars represent the average of five biological replicates (where each replicate consists of a pool of four plants) ±SE. Significant differences between plants grown in control 'C' and drought 'D' conditions, as determined by Student's *t*-test, are indicated by asterisks (***$P \leq 0.001$).

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These results were supported by the increased expression of some of the SL biosynthetic genes (Fig. 2). In the shoot under drought, expression of *D27* and the *MAX1* homologs *Os01g0700900*, *Os01g0701400*, *Os02g0221900*, and *Os06g0565100* was up-regulated. In the root, expression of *D10* and *Os01g0700900* was up-regulated. Of the genes tested, only *D27* was expressed more highly in the shoot than in the root.

Effect of drought on the survival rate of rice SL mutants

The induction of SL biosynthesis under drought could imply that SLs are involved in the adaptation to drought. To explore their contribution to drought tolerance, we analysed how drought affects rice SL mutants. For this purpose, we performed a drought survival assay. The SL-deficient *d10* and *d17* and SL-insensitive *d3* showed significantly higher survival rates (~85, 75, and 45%, respectively), compared with the wild type (20%). In contrast, none of the *d27* plants survived (Fig. 3A, B).

To rule out the possibility that the difference in drought tolerance is due to differences in plant architecture and/or biomass between the mutants, which may cause differences in pot water availability, *d27* and *d10* were analysed in a pooled experiment, in which these two genotypes were grown in the same container. Also under these conditions, the *d27* mutant is more drought sensitive than *d10* (Supplementary Fig. S3).

As water loss by transpiration is one of the most important parameters contributing to drought sensitivity, we measured water loss rates of detached wild-type and SL mutant leaves. The detached leaves of *d10*, *d17*, and *d3* lost water more slowly than wild-type leaves, but *d27* leaves evaporated water significantly faster (Fig. 3C). In addition to this, we examined the stomatal density in one of the drought-resistant *d*-mutants (*d10*) and the drought-sensitive *d27*. Stomatal density was significantly lower in *d10* when compared with the wild type, while stomata size of *d10* showed a tendency to be larger; however, this was not significant. Also, no differences were observed for *d27* (Fig. 4).

ABA content in rice SL mutants

To study if the above-described differences in drought survival, and transpiration rate, between the rice genotypes are linked with differences in ABA levels in the leaf, we quantified ABA in rice SL mutants and the wild type under control conditions and at several time points after water withholding to create different levels of drought.

Under control conditions, ABA leaf content was significantly higher in *d10* and *d17* (1.5-fold) when compared with wild-type plants, but did not differ significantly between *d27* and the wild type (Fig. 5A). A tendency for increased ABA levels was observed for *d3*, although this was not significant. The trend for root ABA in control conditions was the same (data not shown). ABA content was also measured in leaf material that was harvested 2, 6, and 10 d after water withholding. After 2 d, we observed a higher increase in ABA levels in *d10*, *d17*, and *d3* (1.2- to 1.3-fold) than in

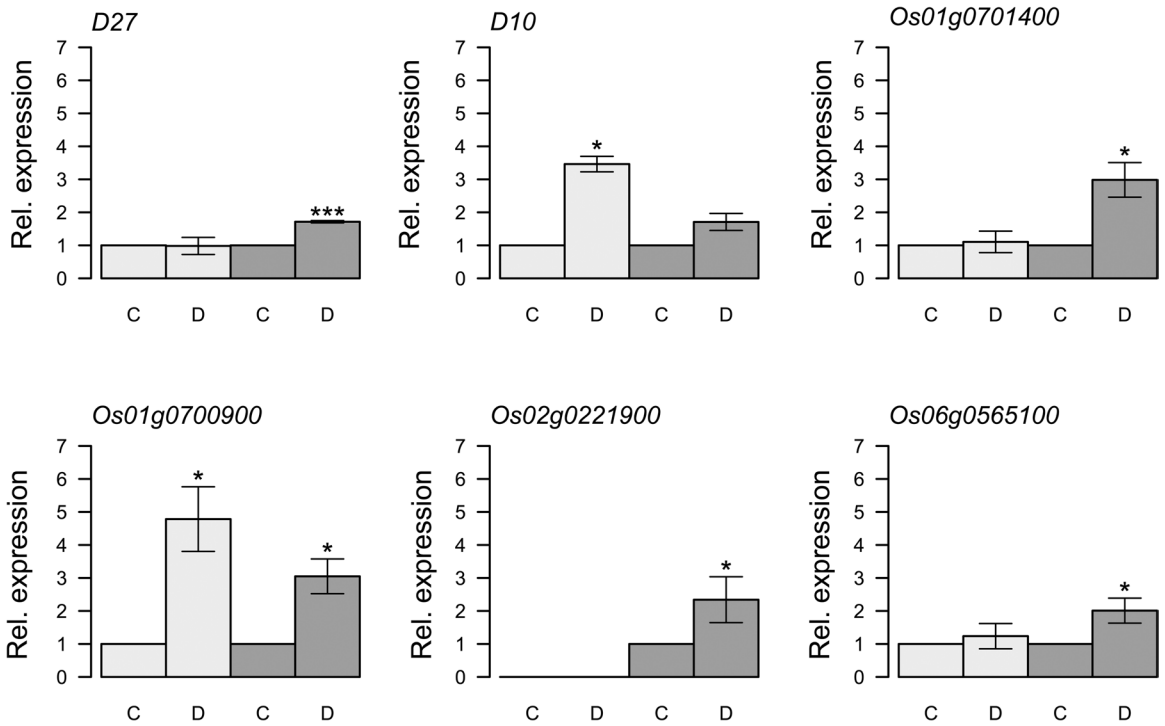

Fig. 2. The effect of mild drought on the expression level of SL biosynthetic genes in rice. The expression level of rice SL biosynthetic pathway genes was measured in root (light grey) and shoot (dark grey). Long-term mild drought (35–45% field capacity) was applied during 9 d (Experiment 1). The *D17* gene was not expressed at detectable levels. Significant differences between control 'C' and drought 'D' treatments in the different tissues, as determined by Student's *t*-test, are indicated by asterisks (*$P \leq 0.05$, ***$P \leq 0.001$). Bars represent the mean of five biological replicates (each replicate consisting of a pool of four plants) ±SE.

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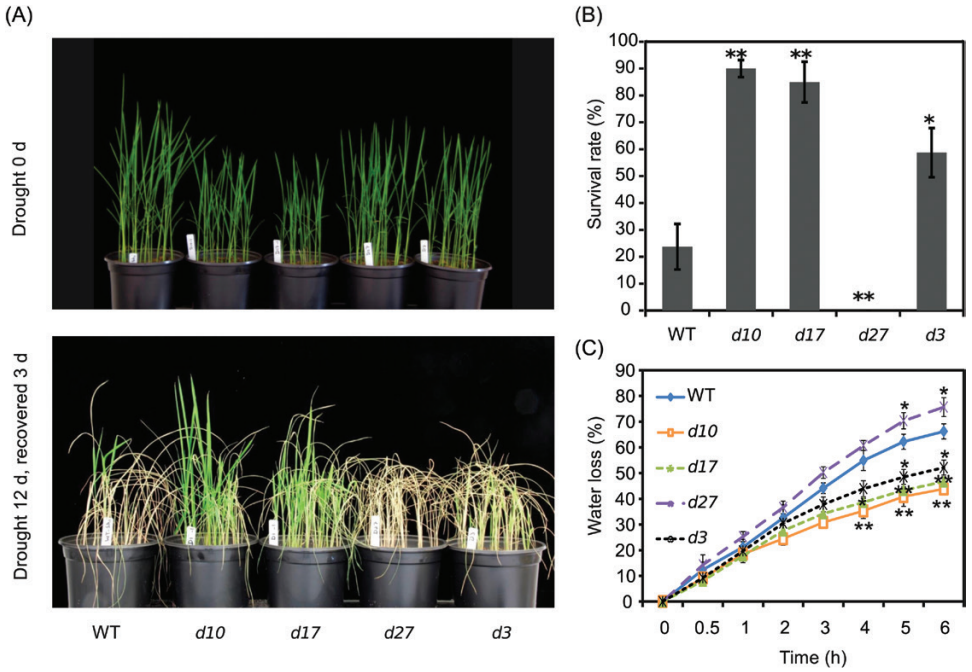

Fig. 3. Plant survival and detached leaf water loss rates of SL-deficient and -insensitive rice mutants and their wild type. (A) The phenotype of five-leaf stage plants before stress and after 12 d of water withholding (strong drought) followed by re-watering and 3 d of recovery (Experiment 2). (B) Survival rate after recovery (five independent biological replicates, each pot containing 16 plants) (Experiment 2). (C) Water loss rate of detached flag leaves (n=9). Bars represent the mean ±SE. Significant differences between mutants and wild-type plants as determined by Student's t-test are indicated by asterisks (*P≤0.05, **P≤0.01).

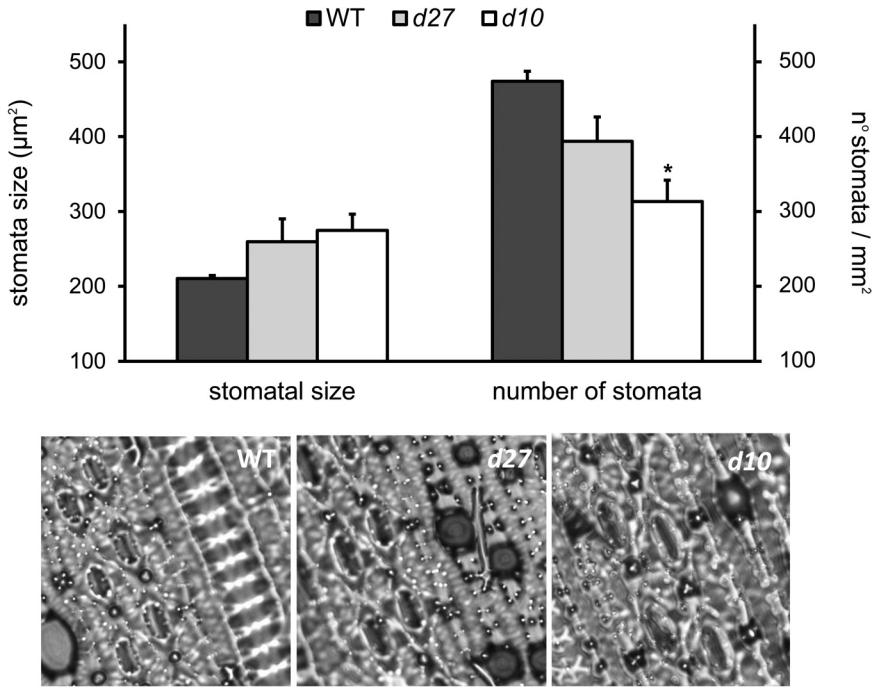

Fig. 4. Stomatal density and size in wild-type, *d10*, and *d27* plants. Barplots show the stomatal size and density from 6-week-old plants in the leaf abaxial side. Bars represent the mean ±SE. An asterisk represents a significant difference compared with the wild type after a pairwise Student t-test (*P<0.05). Microscopy pictures taken for the measurements can be seen at the bottom.

the wild type. After 6 d of water withholding, ABA content was no longer significantly different between the lines but still showed a similar trend to after 2 d of water withholding. After 10 d of drought, ABA content was again higher in *d10*, *d17*, and *d3* (1.1-fold); however, in *d27* ABA levels were significantly lower (0.90-fold), when compared with the wild type (Fig. 5B). To study whether these subtle changes in ABA levels under drought correlate with gene expression, we measured the expression of two ABA biosynthetic genes, *NCED3* and *NCED4*. After 6 d of drought, the expression of

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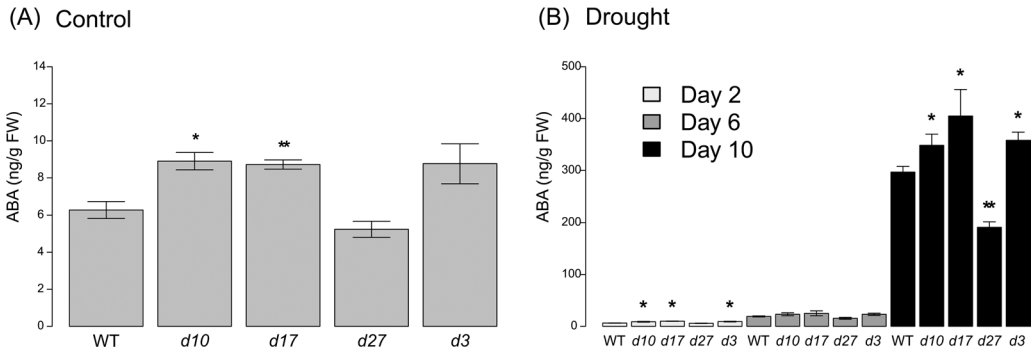

Fig. 5. ABA content in shoots of SL-deficient and -insensitive rice mutants and their wild type. ABA content was measured in the Shiokari wild type (WT), *d10*, *d17*, *d27*, and *d3* mutants by UPLC-MS/MS (Experiment 3). (A) Shoot ABA in five-leaf stage rice plants grown under non-stressed conditions (normal). (B) From the fifth leaf stage onwards, plants were no longer watered (water withholding) and shoot ABA was measured after 2, 6, and 10 d. Bars represent the mean ±SE (n=3). Significant differences between mutants and wild-type plants, as determined by a pairwise comparison Student's t-test, are indicated by asterisks (*P≤0.05, **P≤0.01).

both biosynthetic genes was lower in *d3* and *d17*, while it was higher in *d27*, compared with the wild type (Supplementary Fig. S4). This might be the result of negative feedback on gene expression by the higher ABA levels in *d3* and *d17*, and positive feedback in *d27* by the lower ABA levels (Fig. 5A, B). However, expression of *NCED3* and *NCED4* in *d10* remained similar to that in the wild type (Supplementary Fig. S4). We also tested the expression of two ABA catabolic genes, *CYP707A-2* and *CYP707A-3*, but we did not find any differences from the wild type (data not shown).

Taken together, the results suggest that SL deficiency in rice might stimulate ABA accumulation in the shoot, and that D27 is involved in this, especially under drought conditions. However, the underlying mechanism cannot be explained by changes in the expression level of the above-described genes.

Constitutive overexpression of OsD27 *in rice results in increased levels of ABA*

Because the lower ABA level in *d27* is in contrast to the higher ABA level observed in the other SL biosynthetic/signalling mutants, we addressed the possible function of *OsD27* in ABA formation. For this purpose, we analysed shoot ABA levels in two independent *OsD27* overexpression lines (*D27-OE1* and *D27-OE2*), under both control and drought conditions. The lines did not show morphological changes when compared with their wild type (Supplementary Fig. S5A). *D27* overexpression in these lines was confirmed by qRT-PCR and was between 1000- and 2300-fold higher than for the corresponding wild type (Supplementary Fig. S5B).

Under control conditions, shoot ABA content was significantly increased in both *OsD27* overexpression lines *D27-OE1* and *D27-OE2* (~96% and 32%, respectively) compared with wild-type plants (Fig. 6A), while ABA content in *d27* did not differ from that of the wild type. After 10 d of water withholding, only one of the *OsD27* overexpression lines had a higher ABA level than the wild type, whereas the ABA level in *d27* was significantly lower (Fig. 6B). We also analysed *OsD27* expression in *d10*, *d17*, and *d3* mutants, which contain elevated ABA levels under control conditions, and observed a significantly higher expression in these SL mutants (Fig. 6C). These results show that in these mutant lines ABA content is positively correlated with *D27* gene expression levels. This correlation is also observed when wild-type plants are exposed to drought, during which *D27* expression is induced. All this may be due to a positive feedback of SL deficiency on *OsD27* expression in the SL mutants, which may contribute to their increased ABA levels and hence their increased drought tolerance.

Carotenoid content in the d27 *mutant and* OsD27 *overexpression lines*

In a further attempt to elucidate how D27 affects ABA biosynthesis, we examined the carotenoid composition and content in 2-week-old wild-type plants, the *d27* mutant, and the two *OsD27* overexpression lines, grown under normal conditions. Although all investigated carotenoids (all-*trans*- and 9-*cis*-β-carotene, neoxanthin/violaxanthin, and lutein) showed the tendency to be decreased in the *d27* mutant compared with its wild type, this decrease was only significant for neo-/violaxanthin, lutein, and *trans*-β-carotene. Interestingly, the carotenoid levels were increased (2-fold; Fig. 7) in the *D27-OE2* overexpressing line that only showed a minor increase in ABA content (Fig. 6A).

Discussion

Rice is known to be a large water consumer. Water scarcity is the major limitation for high yield in rain-fed rice and, even under drought conditions, rice yield is severely compromised (Kumar *et al.*, 2008). Exploring ways to produce more rice with less water is essential for food security. This starts with understanding how rice adapts to drought. In the present study, the involvement of SLs in the drought response was assessed in *japonica* rice cv. Shiokari, together with its interaction with the abiotic stress hormone ABA.

The results show that exposure to drought increases the expression of some of the SL biosynthetic genes in root and shoot and that this results in increased SL accumulation in roots. The increase in SL production was more pronounced during a period of stable mild drought with a 10-fold increase

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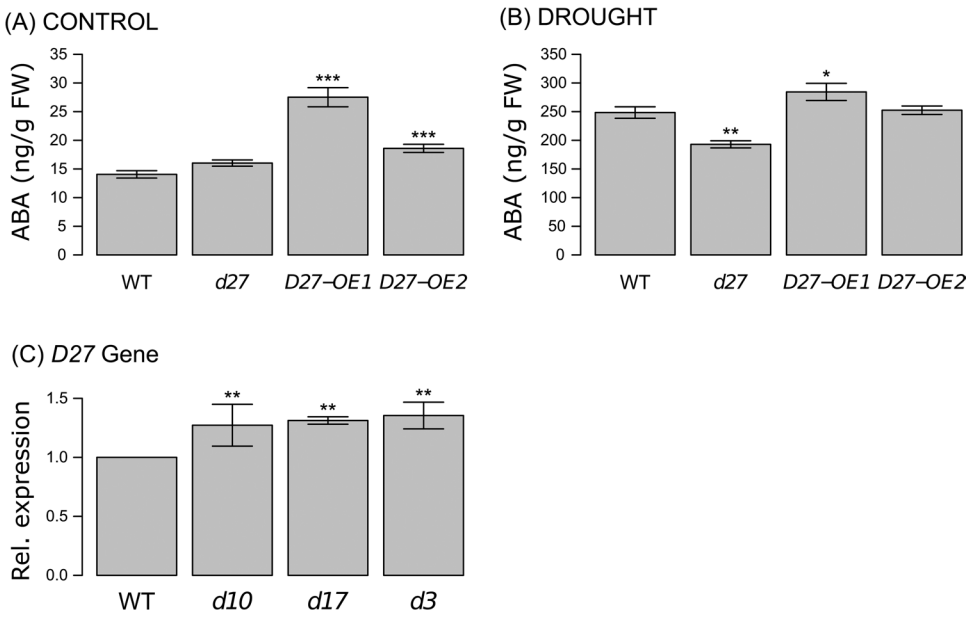

Fig. 6. ABA and *D27* expression levels in shoots of *d27* mutant and *D27*-overexpressing plants, and their wild type. (A and B) ABA content was measured in shoots of 24-day-old wild type (WT), *d27*, and *D27* overexpression lines (*D27-OE1* and *D27-OE2*, both T_4 generation) (Experiment 4) grown under (A) normal conditions and (B) after 10 d of water withholding (strong drought). (C) *OsD27* expression in shoot material from the wild type (WT) and *d10*, *d17*, and *d3* mutants grown under control conditions. Bars represent the mean ±SE from six (A, B) and three (C) biological replicates. Significant differences between mutants, overexpression lines, and the wild type, as determined by a pairwise comparison Student's *t*-test, are indicated by asterisks (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

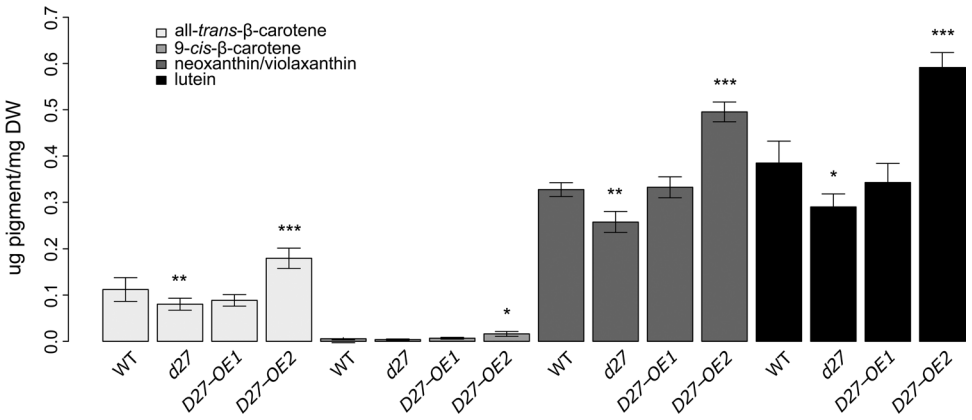

Fig. 7. Carotenoid content in *d27* mutant and *D27*-overexpressing plants (T_4 generation seeds), and their wild type. Carotenoid contents in 2-week-old rice seedlings were quantified by HPLC. Asterisks represent a significant difference compared with the wild type after a pairwise Student's *t*-test (*$P<0.05$, **$P<0.01$, ***$P<0.001$). Values are means ±SE ($n=8$).

in 38-day-old plants (Fig. 1A, B) compared with a 2-fold increase in 25-day-old plants (Supplementary Fig. 7A, B) than under an increasing drought stress. In tomato, Ruiz-Lozano *et al.* (2016) also reported an increase in SLs during drought, but only when plants are colonized with AMF. Perhaps, AMF colonization improved the plant's water uptake and alleviated the stress imposed by water shortage, transforming it from a severe into a mild form of drought stress. In another study using tomato, the expression of the SL biosynthetic gene *Slccd8* was also found to be increased during drought in shoot (Visentin *et al.*, 2016). In contrast to the above findings, it was shown in Lotus that water deficit caused by osmotic stress, resulting from a treatment with polyethylene glycol (PEG), inhibited SL accumulation in roots (Liu *et al.*, 2015). It can be argued, however, that these two forms of drought stress are not comparable in terms of drought severity and adaptation time.

It is well known that different types of drought provoke a different repertoire of plant responses inherent to the different strategies that plants use for their survival during these conditions (Pinheiro and Chaves, 2011). During longer periods of mild drought, stomata remain open, allowing photosynthesis, and plants have the possibility to adapt their architecture. For rice it has been reported that upon drought, plants reduce their leaf production and leaf area, and suppress tillering (Bouman and Tuong, 2001). The latter response is a typical response attributed to the SLs and is in line with our observation of increased root SL levels upon exposure to drought. Besides this, SLs may also be involved in root architectural changes that occur during drought. At the same

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time, drought also induced the expression of SL biosynthetic genes, particularly *D27*, in the shoot (Fig. 2), suggesting that SL production is also increased in above-ground tissues. However, it is unclear which role shoot-based SLs play under these conditions.

During the more severe drought conditions that follow mild spells of drought, stomata need to be closed in order to prevent further water losses. Photosynthesis will be compromised, and the plant should no longer invest in (root) growth. For the latter response, a decrease in SL production and an increase in ABA production are expected. Indeed, in the present study, it is observed that while mild drought resulted in a 10-fold induction of SL production, exposure to more severe drought conditions only led to a 2- to 3-fold increase in SL production (Supplementary Fig. S7). It could be that even longer periods of severe drought will shut down root-based SL biosynthesis completely. This situation would be similar to what is observed in tomato (Visentin *et al.*, 2016) where the expression of SL biosynthesis genes in the root was shut down during the drought, which was suggested to be a prerequisite to allow rapid accumulation of ABA in the shoot.

The effect that SL deficiency/insensitivity had on ABA levels in the SL mutants was reflected in their drought survival rates in dry down assays, where the higher ABA-producing lines *d10*, *d17*, and *d3* were more drought tolerant and the low ABA-producing line *d27* was more drought susceptible when compared with wild-type plants (Fig. 3). These differences correlated with their water loss rates as observed in detached leaf assays. Also, other studies showed that water loss is regulated by stomatal aperture through ABA levels (Franks and Farquhar, 2001; Hwang *et al.*, 2010). Another factor determining the transpiration rate is the stomatal density of the leaves. Indeed, it was observed that the stomatal density of *d10* was lower when compared with its wild type, and thus may have contributed to its improved drought tolerance. The positive effect of a lower stomatal density in *d10* on drought tolerance is also found in Arabidopsis (Ha *et al.*, 2014). However, the fact that the stomatal density in the drought-sensitive *d27* is not higher when compared with the wild type suggests that its increased transpiration rate is due to an ABA-mediated increase in stomatal aperture (Fig. 4). These results make it more likely that the lower ABA levels in *d27*, and the higher ABA levels in *d10* during drought, are indeed contributing to the differences in drought tolerance, perhaps through a difference in stomatal closure time.

In contrast to our observations, drought tolerance in the SL biosynthetic and signalling mutants *max4*, *max3*, and *max2* in Arabidopsis (Bu *et al.*, 2014; Ha *et al.*, 2014) and in tomato and *Lotus* SL-depleted plants (Liu *et al.*, 2015; Ruiz-Lozano *et al.*, 2016; Visentin *et al.*, 2016) was reported to be decreased. Interestingly, the increased drought sensitivity in these Arabidopsis mutants was explained by a reduced sensitivity to ABA compared with wild-type plants. In the study by Bu *et al.* (2014), where the drought sensitivity phenotype was confined to *max2*, ABA levels in the wild type and *max2* were similar under control and drought conditions. Also in tomato and lotus, SL deficiency had a different effect on ABA levels from that in rice. ABA levels in the SL-deficient tomato mutant *slccd8* were reduced (Torres-Vera *et al.*, 2014), and Liu and colleagues showed that in the *CCD7*RNAi lotus line, a combination of phosphate starvation and PEG treatment also resulted in lower ABA levels than in the wild type. Currently, we do not understand the underlying mechanism responsible for the observed differences in ABA levels in rice SL mutants on the one hand and Arabidopsis, tomato, and lotus on the other hand. A genome-wide expression profiling approach using these plant species may perhaps reveal the explanation for this difference.

The reduced ABA level observed in the rice *d27* mutant, when compared with the other *d*-mutants, suggests that D27 may be involved in the regulation of ABA biosynthesis. ABA levels in the shoot of our two independent constitutive *OsD27* overexpression lines were indeed higher than in the wild type. If D27 indeed regulates ABA levels, the increased levels of *D27* expression in *d10*, *d17*, and *d3*, possibly resulting from SL feedback up-regulation (Arite *et al.*, 2007), could explain the higher ABA levels. The even more pronounced increase in the content of ABA in these mutants under drought is probably caused by the simultaneous high expression of *D27* together with the other well-described ABA biosynthesis genes that are specifically induced during this condition (Nambara and Marion-Poll, 2005). However, we cannot exclude that SL deficiency also influences other genes in the carotenoid/ABA pathway. Therefore, it would be of interest also to study *D27* overexpression in other plants, to explore whether the effect that it has on ABA levels in rice is occurring throughout the plant kingdom or is specific for this plant species.

Interestingly, phylogenetic analysis of SL biosynthesis and signalling genes shows that *D27* is highly conserved across the entire green lineage, whereas *CCD7*, *CCD8*, and SL signalling genes are far less conserved, or not even detected in the Chlorophytes (Delaux *et al.*, 2012; Ruyter-Spira and Bouwmeester, 2012). Considering that ABA is already produced in the Chlorophytes (Hartung, 2010), and SLs seem to be a later addition to the repertoire of plant hormones, it can be hypothesized that *D27* has a more ancient role, perhaps in regulating ABA biosynthesis.

The underlying mechanism by which D27 controls ABA levels in rice is currently not known. *In vitro* experiments demonstrated that D27 is not directly involved in the formation of the ABA precursors 9-*cis* violaxanthin or 9'-*cis*-neoxanthin from their all-*trans* precursors (Bruno and Al-Babili, 2016). However, D27 was found to isomerize α-carotene and β,β-cryptoxanthin (Bruno and Al-Babili, 2016). Perhaps, the absence of undefined end-products resulting from these side branches of the carotenoid pathway may somehow impact on ABA biosynthesis. Another possibility is that, considering that D27 acts on multiple substrates from the same pathway, D27 overexpression or D27 inactivity will have a large impact on the flux of the carotenoid pathway, ultimately resulting in altered levels of its final products such as ABA.

Although ABA was increased in both *D27* overexpression lines, ABA levels in line *D27-OE1* were higher than in *D27-OE2*. This correlated with a higher expression level of *NCED3* in *D27-OE1* (Supplementary Fig. S6). However, in its turn, line *D27-OE2* contained higher

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carotenoid levels (neo-/violaxanthin) than *D27-OE1* (Fig. 7). The latter was also observed when an ABA biosynthesis gene, β-carotene hydroxylase (*DSM2* or *BCH*), was overexpressed in rice (Du *et al.*, 2010), showing that overexpression of ABA biosynthesis genes does not always lead to increased levels of ABA but may also lead to accumulation of pathway intermediates through an as yet unknown mechanism. Lutein was also found to be increased in line *D27-OE2* when compared with the wild type. Interestingly, the concentration of lutein and neo-/violaxanthin was found to be decreased in leaves of *d27*. Considering that lutein is the end-product of the β,ε branch of carotenoids and ABA the end-product of the β,β branch, it is striking that besides β-carotene, α-carotene has also recently been identified as a substrate for D27 (Bruno and Al-Babili, 2016).

Our findings show that SLs play an active role in drought physiology. Moreover, our results suggest a connection between the SL and ABA pathways, and a crucial role for *D27* in determining ABA and SL content in rice, indicating that this gene may be a promising target for drought improvement in crops. Further studies on how D27 fine-tunes the level of ABA and SLs in different stress responses, as well as in developmental processes, should increase our understanding of the role of D27 in regulating SL and ABA homeostasis and hence in determining drought tolerance in plants.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Shared biosynthesis pathway of strigolactone (SL) and abscisic acid (ABA).

Fig. S2. Biomass (A) and ABA content (B) from rice plants under drought and control conditions (Experiment 1).

Fig. S3. Pooled drought survival assay of the *d27* and *d10* SL biosynthetic mutant lines (Experiment 5).

Fig. S4. ABA biosynthetic *OsNCED3* and *OsNCED4* gene expression.

Fig. S5. Phenotypic and transcriptional comparison of *D27* overexpressor and *d27* mutant plants, together with their wild type.

Fig. S6. *NCED3* expression levels in shoots of *D27*-overexpressing plants and their wild type (Experiment 4).

Fig. 7. Effect of strong drought on SL content in rice root extracts.

Table S1. Primer sequences used for gene expression analysis and overexpression lines.

Acknowledgements

We thank Professors J. Li and Y. Wang for kindly providing the rice *OsD27* overexpression lines. We thank Francel Verstappen for his technical support at WUR in the Netherlands. This work was supported by the Higher Education Commission (HEC) of Pakistan to IH, Netherlands Organization for Scientific Research (NWO; Equipment grant, 834.08.001 to HJB), and King Abdullah University of Science and Technology (KAUST) and the EU (METAPRO; FP7 KBBE-2009-3-1-01). We acknowledge a private donor who supported BAJ's work via the Wageningen University Fund.

References

Abe S, Sado A, Tanaka K, Kisugi T, Asami K, Ota S, Kim HI, Yoneyama K, Xie X, Ohnishi T. 2014. Carlactone is converted to carlactonoic acid by MAX1 in Arabidopsis and its methyl ester can directly interact with AtD14 in vitro. Proceedings of the National Academy of Sciences, USA **111,** 18084–18089.

Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435,** 824–827.

Al-Babili S, Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid-derived plant hormone. Annual Review of Plant Biology **66,** 161–186.

Alder A, Jamil M, Marzorati M, Bruno M, Vermathen M, Bigler P, Ghisla S, Bouwmeester H, Beyer P, Al-Babili S. 2012. The path from β-carotene to carlactone, a strigolactone-like plant hormone. Science **335,** 1348–1351.

Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J. 2007. DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. The Plant Journal **51,** 1019–1029.

Booker J, Auldridge M, Wills S, McCarty D, Klee H, Leyser O. 2004. MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Current Biology **14,** 1232–1238.

Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O. 2005. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Developmental Cell **8,** 443–449.

Borghi L, Kang J, Ko D, Lee Y, Martinoia E. 2015. The role of ABCG-type ABC transporters in phytohormone transport. Biochemical Society Transactions **43,** 924–930.

Bouman B, Tuong TP. 2001. Field water management to save water and increase its productivity in irrigated lowland rice. Agricultural Water Management **49,** 11–30.

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH. 2003. Secondary metabolite signalling in host–parasitic plant interactions. Current Opinion in Plant Biology **6,** 358–364.

Bouwmeester HJ, Roux C, Lopez-Raez JA, Bécard G. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. Trends in Plant Science **12,** 224–230.

Brewer PB, Yoneyama K, Filardo F, Meyers E, Scaffidi A, Frickey T, Akiyama K, Seto Y, Dun EA, Cremer JE. 2016. LATERAL BRANCHING OXIDOREDUCTASE acts in the final stages of strigolactone biosynthesis in Arabidopsis. Proceedings of the National Academy of Sciences, USA **113,** 6301–6306.

Britton G, Liaaen-Jensen S, Pfander H, eds. 1995. Carotenoids, Vol. 1B: Spectroscopy. Basel: Birkhäuser, 13–63.

Bruno M, Al-Babili S. 2016. On the substrate specificity of the rice strigolactone biosynthesis enzyme DWARF27. Planta **243,** 1429–1440.

Bruno M, Hofmann M, Vermathen M, Alder A, Beyer P, Al-Babili S. 2014. On the substrate- and stereospecificity of the plant carotenoid cleavage dioxygenase 7. FEBS Letters **588,** 1802–1807.

Bruno M, Vermathen M, Alder A, Wüst F, Schaub P, van der Steen R, Beyer P, Ghisla S, Al-Babili S. 2017. Insights into the formation of carlactone from in-depth analysis of the CCD8-catalyzed reactions. FEBS Letters **591,** 792–800.

Bu Q, Lv T, Shen H, *et al*. 2014. Regulation of drought tolerance by the F-box protein MAX2 in Arabidopsis. Plant Physiology **164,** 424–439.

Burbidge A, Grieve TM, Jackson A, Thompson A, McCarty DR, Taylor IB. 1999. Characterization of the ABA-deficient tomato mutant notabilis and its relationship with maize Vp14. The Plant Journal **17,** 427–431.

Cardoso C, Zhang Y, Jamil M, Hepworth J, Charnikhova T, Dimkpa SO, Meharg C, Wright MH, Liu J, Meng X. 2014. Natural variation of rice strigolactone biosynthesis is associated with the deletion of two MAX1 orthologs. Proceedings of the National Academy of Sciences, USA **111,** 2379–2384.

Cheng WH, Endo A, Zhou L, *et al*. 2002. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. The Plant Cell **14,** 2723–2743.

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Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. 1966. Germination of Witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. Science **154,** 1189–1190.

Delaux PM, Xie X, Timme RE, Puech-Pages V, Dunand C, Lecompte E, Delwiche CF, Yoneyama K, Bécard G, Séjalon-Delmas N. 2012. Origin of strigolactones in the green lineage. New Phytologist **195,** 857–871.

de Saint Germain A, Clavé G, Badet-Denisot MA, *et al*. 2016. An histidine covalent receptor and butenolide complex mediates strigolactone perception. Nature Chemical Biology **12,** 787–794.

Du H, Wang N, Cui F, Li X, Xiao J, Xiong L. 2010. Characterization of the beta-carotene hydroxylase gene DSM2 conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice. Plant Physiology **154,** 1304–1318.

Finkelstein R. 2013. Abscisic acid synthesis and response. The Arabidopsis Book **11,** e0166.

Franks PJ, Farquhar GD. 2001. The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. Plant Physiology **125,** 935–942.

Giday H, Kjaer KH, Fanourakis D, Ottosen CO. 2013. Smaller stomata require less severe leaf drying to close: a case study in *Rosa hydrida*. Journal of Plant Physiology **170,** 1309–1316.

Gomez-Roldan V, Fermas S, Brewer PB, *et al*. 2008. Strigolactone inhibition of shoot branching. Nature **455,** 189–194.

Ha CV, Leyva-González MA, Osakabe Y, Tran UT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi S, Van Dong N. 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proceedings of the National Academy of Sciences, USA **111,** 851–856.

Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC. 2012. DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. Current Biology **22,** 2032–2036.

Hartung W. 2010. The evolution of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen. Functional Plant Biology **37,** 806–812.

Hiei Y, Ohta S, Komari T, Kumashiro T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. The Plant Journal **6,** 271–282.

Hwang S-G, Chen H-C, Huang W-Y, Chu Y-C, Shii C-T, Cheng W-H. 2010. Ectopic expression of rice OsNCED3 in Arabidopsis increases ABA level and alters leaf morphology. Plant Science **178,** 12–22.

Jamil M, Charnikhova T, Cardoso C, Jamil T, Ueno K, Verstappen F, Asami T, Bouwmeester H. 2011*a*. Quantification of the relationship between strigolactones and *Striga hermonthica* infection in rice under varying levels of nitrogen and phosphorus. Weed Research **51,** 373–385.

Jamil M, Rodenburg J, Charnikhova T, Bouwmeester HJ. 2011*b*. Pre-attachment *Striga hermonthica* resistance of New Rice for Africa (NERICA) cultivars based on low strigolactone production. New Phytologist **192,** 964–975.

Jia K-P, Baz L, Al-Babili S. 2017. From carotenoids to strigolactones. Journal of Experimental Botany **69,** 2189–2204.

Jiang L, Liu X, Xiong G, *et al*. 2013. DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature **504,** 401–405.

Ko D, Helariutta Y. 2017. Shoot–root communication in flowering plants. Current Biology **27,** R973–R978.

Kumar A, Bernier J, Verulkar S, Lafitte H, Atlin G. 2008. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crops Research **107,** 221–231.

Lin H, Wang R, Qian Q, *et al*. 2009. DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. The Plant Cell **21,** 1512–1525.

Liu J, He H, Vitali M, *et al*. 2015. Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: exploring the interaction between strigolactones and ABA under abiotic stress. Planta **241,** 1435–1451.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods **25,** 402–408.

López-Ráez JA, Charnikhova T, Gómez-Roldán V, *et al*. 2008. Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytologist **178,** 863–874.

López-Ráez JA, Kohlen W, Charnikhova T, *et al*. 2010. Does abscisic acid affect strigolactone biosynthesis? New Phytologist **187,** 343–354.

Matusova R, Rani K, Verstappen FW, Franssen MC, Beale MH, Bouwmeester HJ. 2005. The strigolactone germination stimulants of the plant-parasitic Striga and Orobanche spp. are derived from the carotenoid pathway. Plant Physiology **139,** 920–934.

Moise AR, Al-Babili S, Wurtzel ET. 2014. Mechanistic aspects of carotenoid biosynthesis. Chemical Reviews **114,** 164–193.

Nakamura H, Xue YL, Miyakawa T, *et al*. 2013. Molecular mechanism of strigolactone perception by DWARF14. Nature Communications **4,** 2613.

Nambara E, Marion-Poll A. 2005. Abscisic acid biosynthesis and catabolism. Annual Review of Plant Biology **56,** 165–185.

Nisar N, Li L, Lu S, Khin NC, Pogson BJ. 2015. Carotenoid metabolism in plants. Molecular Plant **8,** 68–82.

Pinheiro C, Chaves MM. 2011. Photosynthesis and drought: can we make metabolic connections from available data? Journal of Experimental Botany **62,** 869–882.

Ruiz-Lozano JM, Aroca R, Zamarreño ÁM, Molina S, Andreo-Jiménez B, Porcel R, García-Mina JM, Ruyter-Spira C, López-Ráez JA. 2016. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant, Cell and Environment **39,** 441–452.

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. 2013. The biology of strigolactones. Trends in Plant Science **18,** 72–83.

Ruyter-Spira C, Bouwmeester H. 2012. Strigolactones affect development in primitive plants. The missing link between plants and arbuscular mycorrhizal fungi? New Phytologist **195,** 730–733.

Santner A, Estelle M. 2009. Recent advances and emerging trends in plant hormone signalling. Nature **459,** 1071–1078.

Schwartz SH, Qin X, Zeevaart JA. 2003. Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. Plant Physiology **131,** 1591–1601.

Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR. 1997. Specific oxidative cleavage of carotenoids by VP14 of maize. Science **276,** 1872–1874.

Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JA, Koornneef M, Kamiya Y, Koshiba T. 2000. The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proceedings of the National Academy of Sciences, USA **97,** 12908–12913.

Seto Y, Sado A, Asami K, Hanada A, Umehara M, Akiyama K, Yamaguchi S. 2014. Carlactone is an endogenous biosynthetic precursor for strigolactones. Proceedings of the National Academy of Sciences, USA **111,** 1640–1645.

Shinozaki K, Yamaguchi-Shinozaki K. 2007. Gene networks involved in drought stress response and tolerance. Journal of Experimental Botany **58,** 221–227.

Smith S, Weyers JDB, Berry WG. 1989. Variation in stomatal characteristics over the lower surface of *Commelina communis* leaves. Plant, Cell and Environment **12,** 653–659.

Sorefan K, Booker J, Haurogné K, *et al*. 2003. MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. Genes and Development **17,** 1469–1474.

Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson DC. 2015. SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in Arabidopsis. The Plant Cell **27,** 3143–3159.

Stirnberg P, Furner IJ, Ottoline Leyser HM. 2007. MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. The Plant Journal **50,** 80–94.

Tan BC, Schwartz SH, Zeevaart JA, McCarty DR. 1997. Genetic control of abscisic acid biosynthesis in maize. Proceedings of the National Academy of Sciences, USA **94,** 12235–12240.

Torres-Vera R, García JM, Pozo MJ, López-Ráez JA. 2014. Do strigolactones contribute to plant defence? Molecular Plant Pathology **15,** 211–216.

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Umehara M, Hanada A, Yoshida S, *et al*. 2008. Inhibition of shoot branching by new terpenoid plant hormones. Nature **455,** 195–200.

Visentin I, Vitali M, Ferrero M, Zhang Y, Ruyter-Spira C, Novák O, Strnad M, Lovisolo C, Schubert A, Cardinale F. 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. New Phytologist **212,** 954–963.

Wang L, Wang B, Jiang L, Liu X, Li X, Lu Z, Meng X, Wang Y, Smith SM, Li J. 2015. Strigolactone signaling in Arabidopsis regulates shoot development by targeting D53-like SMXL repressor proteins for ubiquitination and degradation. The Plant Cell **27,** 3128–3142.

Wang Y, Li J. 2011. Branching in rice. Current Opinion in Plant Biology **14,** 94–99.

Wang Z, Chen C, Xu Y, Jiang R, Han Y, Xu Z, Chong K. 2004. A practical vector for efficient knockdown of gene expression in rice (*Oryza sativa* L.). Plant Molecular Biology Reporter **22,** 409–417.

Waters MT, Brewer PB, Bussell JD, Smith SM, Beveridge CA. 2012. The Arabidopsis ortholog of rice DWARF27 acts upstream of MAX1 in the control of plant development by strigolactones. Plant Physiology **159,** 1073–1085.

Waters MT, Gutjahr C, Bennett T, Nelson DC. 2017. Strigolactone signaling and evolution. Annual Review of Plant Biology **68,** 291–322.

Wolters H, Jürgens G. 2009. Survival of the flexible: hormonal growth control and adaptation in plant development. Nature Reviews Genetics **10,** 305–317.

Xie X, Yoneyama K, Yoneyama K. 2010. The strigolactone story. Annual Review of Phytopathology **48,** 93–117.

Yao R, Ming Z, Yan L, *et al*. 2016. DWARF14 is a non-canonical hormone receptor for strigolactone. Nature **536,** 469–473.

Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H. 2007. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta **225,** 1031–1038.

Zhang S, Haider I, Kohlen W, Jiang L, Bouwmeester H, Meijer AH, Schluepmann H, Liu CM, Ouwerkerk PB. 2012. Function of the HD-Zip I gene Oshox22 in ABA-mediated drought and salt tolerances in rice. Plant Molecular Biology **80,** 571–585.

Zhang Y, Haider I, Ruyter-Spira C, Bouwmeester H. 2013. Strigolactone biosynthesis and biology. In: **Bruijn FJd**, ed. Molecular microbial ecology of the rhizosphere, Vol. **1**. Hoboken, NJ: John Wiley & Sons, Inc., 355–371.

Zhang Y, van Dijk AD, Scaffidi A, *et al*. 2014. Rice cytochrome P450 MAX1 homologs catalyze distinct steps in strigolactone biosynthesis. Nature Chemical Biology **10,** 1028–1033.

Zhou F, Lin Q, Zhu L, *et al*. 2013. D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signalling. Nature **504,** 406–410.

Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L. 2006. The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. The Plant Journal **48,** 687–698.

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