

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

Imran Haider^{1, 2, †}, Beatriz Andreo-Jimenez^{1, †}, Mark Bruno³, Andrea Bimbo¹, Kristýna Floková^{1,4}, Haneen Abuauf², Valentine Otang Ntui², Xiujie Guo^{2, #a}, Tatsiana Charnikhova¹, Salim Al-Babili^{2, 3}, Harro J. Bouwmeester^{1, 4, *}, Carolien Ruyter-Spira¹

¹ Laboratory of Plant Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

² Bioactives Lab, Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia

³ Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

⁴ Plant hormone biology group, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

^{#a} Present address: International Institute of Tropical Agriculture, Nairobi, Kenya

[†] These authors contributed equally to this work.

* Author to whom correspondence should be addressed: h.j.bouwmeester@uva.nl, tel. +31 6 16 20387674

Supplementary data

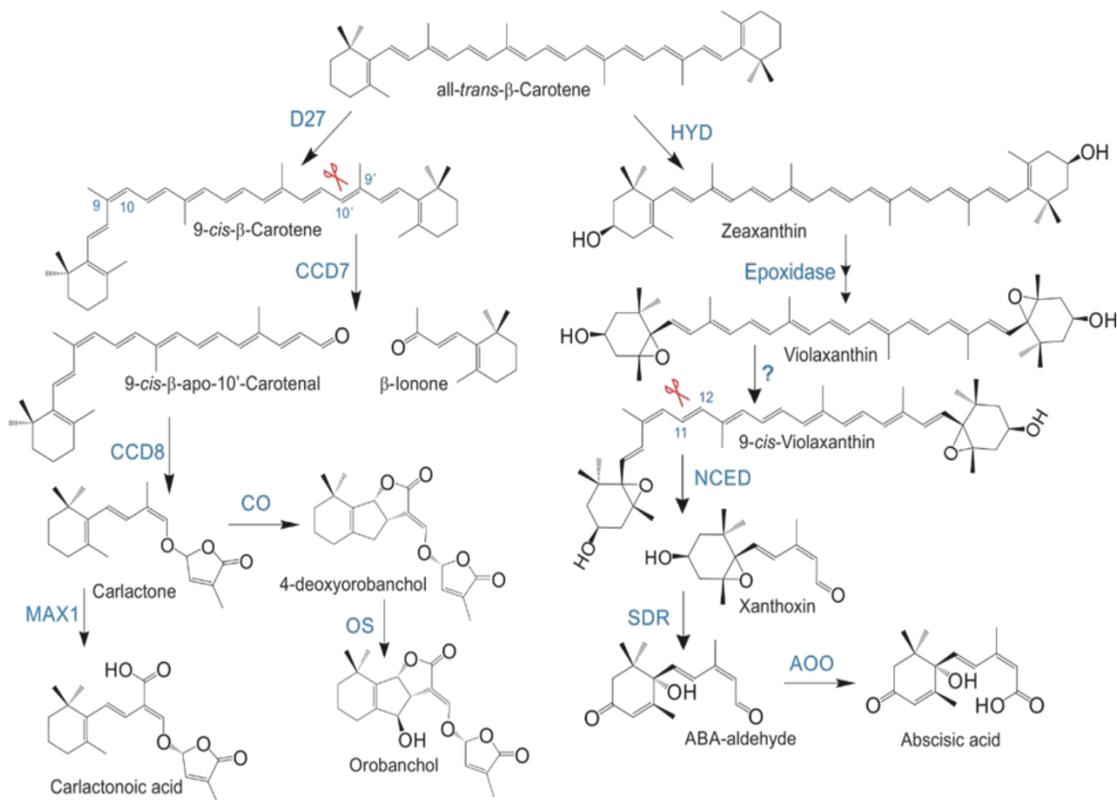


Fig. S1. Shared biosynthesis pathway of strigolactone (SL) and abscisic acid (ABA).

SL biosynthesis: The conversion of *all-trans*- β -carotene to carlactone is catalysed by D27, CCD7 and CCD8, respectively (Alder *et al.*, 2012). Rice MAX1 homolog, CO catalyses the conversion of carlactone into the SL-parent molecule, 4-deoxyorobanchol, which is further cleaved by second rice MAX1-homolog, OS to form orobanchol (Zhang *et al.*, 2014; Al-Babili and Bouwmeester, 2015). *Arabidopsis* MAX1 has also been shown to convert carlactone into carlactonoic acid (Abe *et al.*, 2014).

ABA biosynthesis: The conversion of *all-trans*- β -carotene to xanthoxin is catalysed by HYD, epoxidase and NCED, respectively. *All-trans*-neoxanthin is derived from *all-trans*-violaxanthin. The conversion of *all-trans*-violaxanthin and/or -neoxanthin into the corresponding 9-*cis*-isomer is unidentified. The xanthoxin is further cleaved by SDR and AAO to form ABA (Nambara and Marion-Poll, 2005).

Abbreviations: HYD (β -carotene hydroxylase), NCED (9-*cis*-epoxycarotenoid dioxygenases), SDR (short-chain dehydrogenase/reductase), AAO (abscisic aldehyde oxidase), D27 (DWARF27), CCDs (carotenoid cleavage dioxygenases), MAX1 (MORE AXILLARY GROWTH 1), CO (carlactone oxidase), OS (orobanchol synthase).

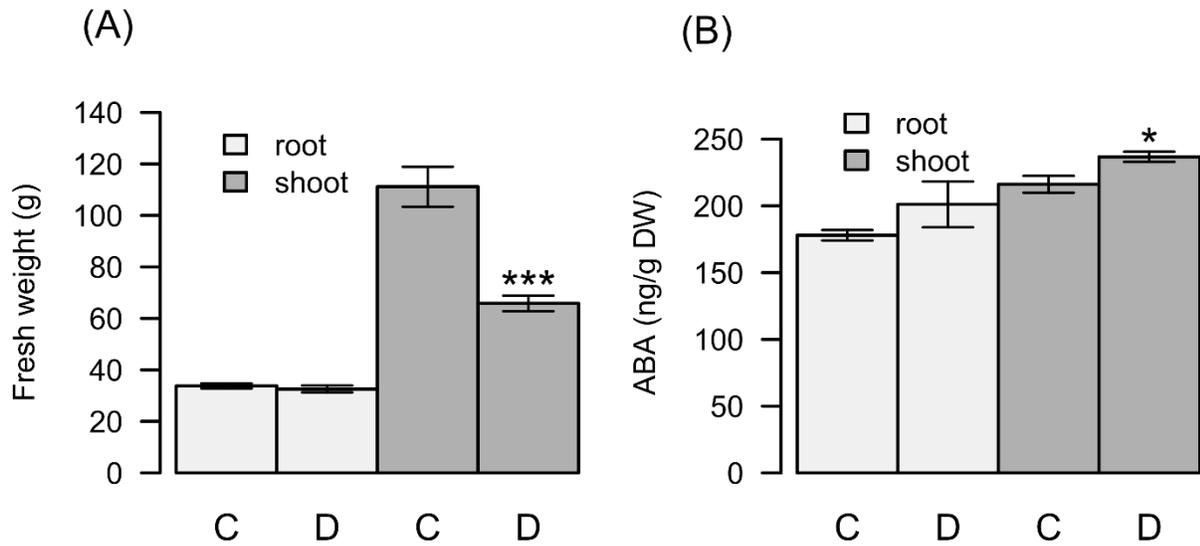


Fig. S2. Biomass (A) and ABA content (B) from rice plants under drought and control conditions (Experiment 1).

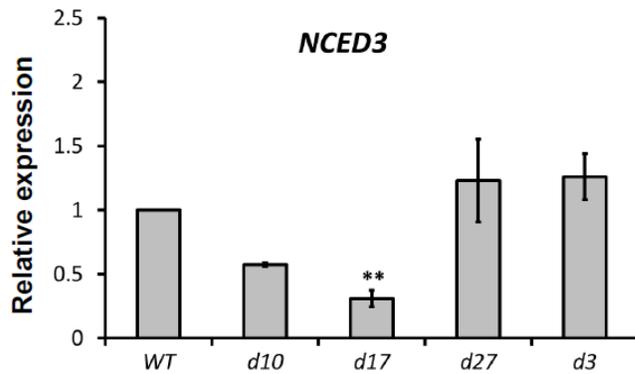
Long term mild drought (35-45 % FC) was applied during 9 d. Data comes from six-week-old plants and is derived from five biological replicates (and each replicate is represented by a pool of four plants). There are significant differences between treatments, control 'C' and drought 'D' as revealed by Student's *t*-test (** $P \leq 0.001$, * $P < 0.05$). Bars represent mean \pm standard error.



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

The phenotype of five-leaf stage *d27* and *d10* mutant plants after 12 d of water withholding (strong drought) and 3 d of re-water recovery in a pooled pot experiment. Eight independent biological replicates.

(A)



(B)

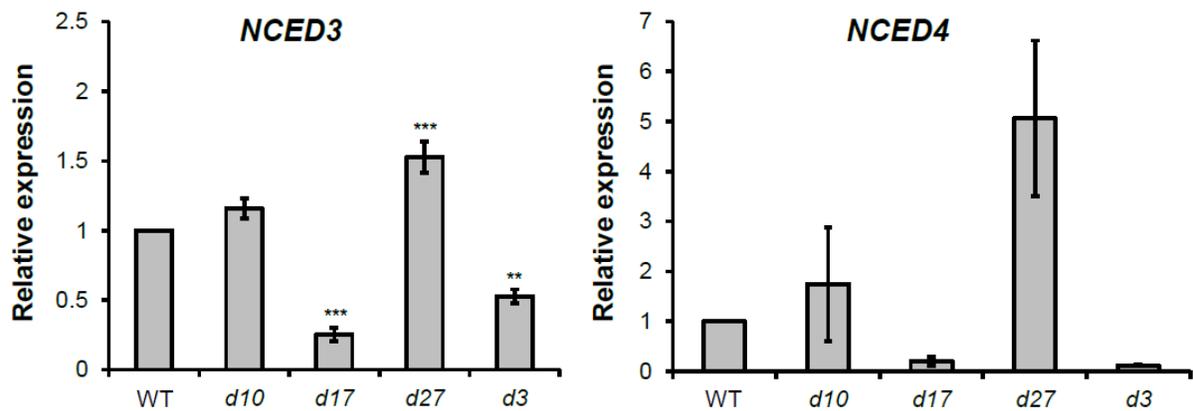


Fig. S4. ABA biosynthetic *OsNCED3* and *OsNCED4* gene expression.

Shoot gene expression for ABA biosynthetic genes were measured before (A) and after (B) 6 days of water withholding (strong drought) in fifth leaf stage plants (Experiment 3). Bars represent mean \pm standard error ($n=3$). Significant differences between mutants and wild-type plants, as determined by a pairwise comparison Student's *t*-test, are indicated by asterisks (** $P \leq 0.01$, *** $P \leq 0.001$).

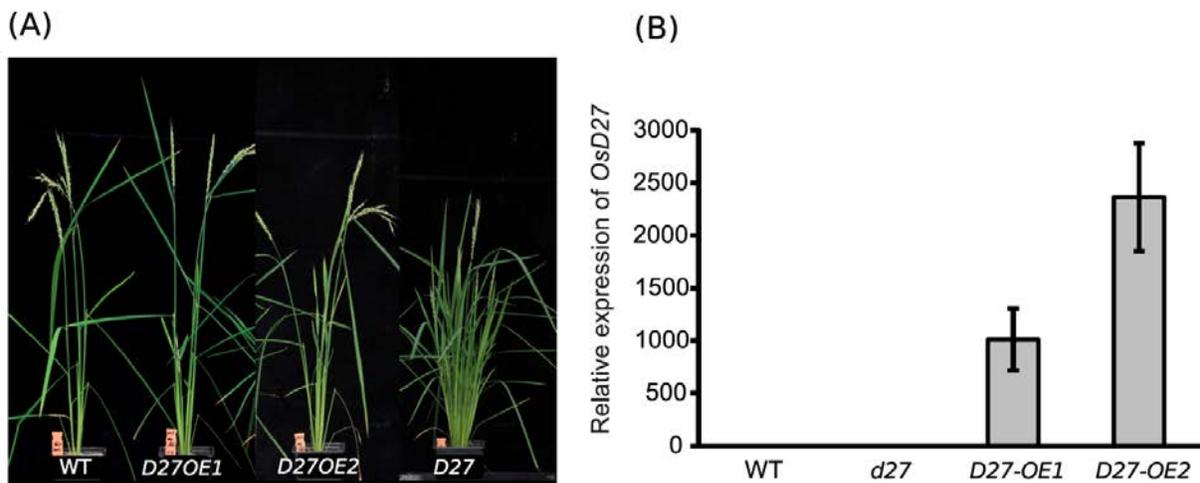


Fig. S5. Phenotypic and transcriptional comparison of *D27* over-expressor - and *d27* mutant plants, together with their wild-type.

(A) Morphology of Shiokari wild-type (WT), *D27* over-expression (*D27-OE1* and *D27-OE2*), and *d27* rice plants at post-anthesis stage. (B) The relative expression level of *OsD27* in 19-days-old plants of the two independent over-expression lines (*D27-OE1* and *D27-OE2*) as compared with WT. Values are normalized with the rice *ubiquitin* gene. Bars represent mean \pm standard error.

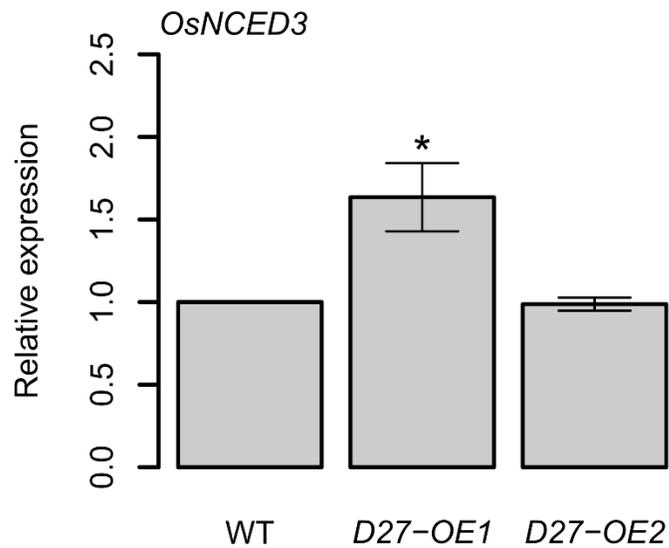


Fig. S6. *NCED3* expression levels in shoots of *D27* over-expressing plants and their wild-type (Experiment 4).

Values are normalized with the rice *ubiquitin* gene. Bars represent mean \pm standard error. Asterisk represents a significant difference with the wild-type after a pairwise Student *t*-test (*, $P < 0.05$).

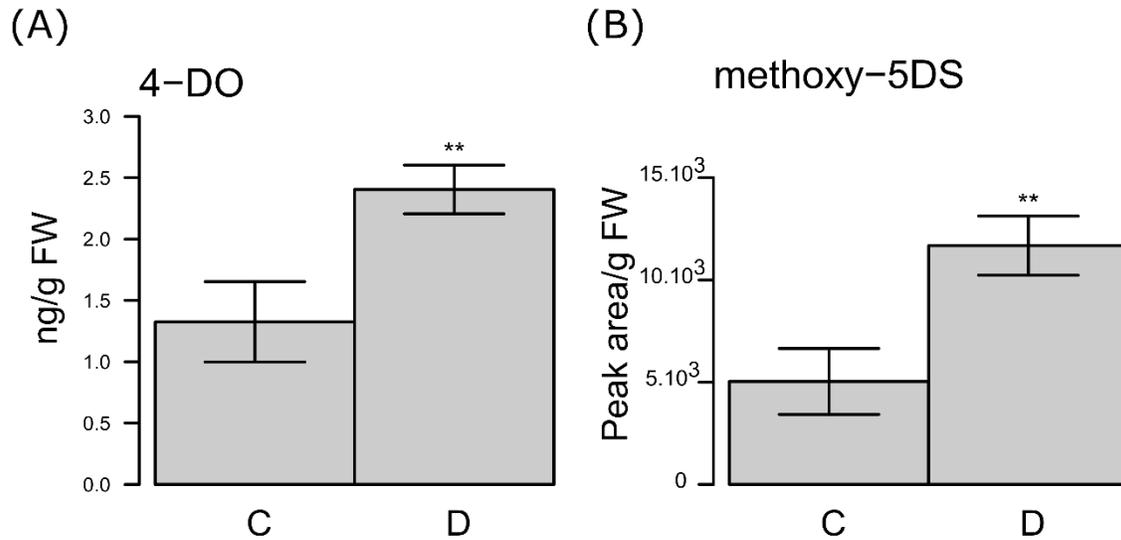


Fig. S7. Effect of strong drought on SLs content in rice root extracts.

Levels of 4-DO: 4-deoxyorobanchol (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. Root collected from 25 days-old rice plants (Experiment 1). Strong drought by water withholding was applied during 11 d. Bars represent the average of six biological replicates \pm standard error. Significant differences between plants grown in control 'C' and drought 'D' conditions, as determined by Student's *t*-test, are indicated by asterisks (** $P \leq 0.01$).

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

Primer name	Sequence
<i>D27-Q-F</i>	5'-TCTGGGCTAAAGAATGAAAAGGA-3'
<i>D27-Q-R</i>	5'-AGAGCTTGGGTCACAATCTCG-3'
<i>D27-OE-F</i>	5'-AAGGATCCATGGAGACCACCACGCTTGT-3'
<i>D27-OE-R</i>	5'-AAACTAGTTCAGATGGAGCAATTCACAC-3'
<i>OsNCED3-Q-F</i>	5'-CCCCTCCCAAACCATCCAAACCGA-3'
<i>OsNCED3-Q-R</i>	5'-TGTGAGCATATCCTGGCGTCGTGA-3'
<i>OsCYP707A-2-F</i>	5'-CTACTGCTGATGGTGGCTGA-3'
<i>OsCYP707A-2-R</i>	5'-CCCATGGCCTTTGCTTTAT-3'
<i>OsCYP707A-3-F</i>	5'-AGTACAGCCATTCCCTGTG -3'
<i>OsCYP707A-3-R</i>	5'-ACGCCTAATCAAACCATTGC-3'
<i>Ubi-F</i>	5'-GCCCAAGAAGAAGATCAAGAAC-3'
<i>Ubi-R</i>	5'-AGATAACAACGGAAGCATAAAAGTC-3'
<i>D10-Q-F</i>	5'-CGTGGCGATATCGATGGT-3'
<i>D10-Q-R</i>	5'-CGACCTCCTCGAACGTCTT-3'
<i>D17-Q- F</i>	5'-TCCACAGGATGTTTGGTTACA-3'
<i>D17-Q-R</i>	5'-GTAGCTTGGGTTTATCGCCG-3'
<i>Os01g0701400-Q- F</i>	5'-TGCAGACCAAGTTCCCCTAC-3'
<i>Os01g0701400-Q-R</i>	5'-CACCCATGTTCCCTTTGG-3'
<i>Os01g0700900-Q-F</i>	5'-GAGTTGTGCAAGGAAGTGGG-3'
<i>Os01g0700900-Q-R</i>	5'-GTCCACCTCGAACCCTTGT-3'
<i>Os06g0565100-Q- F</i>	5'-CTCTCCACCAGAAGGGCCTC-3'
<i>Os06g0565100-Q- R</i>	5'-GAGATGATCGTGTTCCTCATCG-3'
<i>Os02g0221900-Q- F</i>	5'-AGGTCATCAAGGAGGCCATG-3'
<i>Os02g0221900-Q- R</i>	5'-CACACGTACGTCCCCTTTG-3'