Arabidopsis phenotyping reveals the importance of ALCOHOL DEHYDROGENASE and PYRUVATE DECARBOXYLASE for aerobic plant growth

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Supplementary Information

Color channel	Feature Name	Туре	feature size	calculation
Red	haar_6	Block	8	Sum
Red	haar_8	Block	8	Sum
Red	haar_9	Block	8	Sum
Red	haar_10	Block	8	Sum
Red	haar_11	Block	8	Sum
Red	haar_12	Block	8	Sum
Red	haar_13	Block	8	Sum
Red	haar_17	Line	1x3	Sum
Red	haar_17	Line	4x12	Sum
Green	Haar_5	Block	15	Sum
Green	Haar_5	Block	30	Sum
Green	Haar_10	Block	15	Sum
Green	Haar_10	Block	30	Sum
Green	Haar_12	Block	15	Sum
Green	Haar_12	Block	30	Sum

Supplemental Table S1: List of haar-like features used for the machine-learning algorithm from the OpenCV library.

 Supplemental Table S2: list of texture features used following Laws 1980 (Laws, K. I. Textured image segmentation (No. USCIPI-940). University of Southern California Los Angeles Image Processing INST. (1980) taken from the OpenCV library.

Color channel	Feature name	Block size
Red	E5E5	15x15
Red	E5L5	15x15
Red	E5R5	15x15
Red	L5S5	15x15
Red	L5R5	15x15
Red	L5S5	15x15
Red	R5E5	15x15
Red	R5L5	15x15
Red	R5R5	15x15
Red	R5S5	15x15
Red	S5E5	15x15
Red	S5L5	15x15
Red	S5R5	15x15
Red	\$5\$5	15x15

Supplemental Table S3. List of primers.

Gene (AGI)	Forward primer	Reverse primer	
At4g05320	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAG	
(UBQ10)		Т	
At1g77120 (ADH)	CAATTGCTGGACTGCAAAGGTG	AGCAACTCTTTGCTCGTATCATCC	
At4g33070 (PDC1)	CAATTGCTGGACTGCAAAGGTG	AGCAACTCTTTGCTCGTATCATCC	
AT5G54960 (PDC2)	CCCCAAATCCGCAGTAGAGT	CCTCAAGGGGACACACATTT	
AT1G01480	ATGGGTCTTGCAGAGAATCAGC	ACCTTCAAGGGTGCAAATAGAAGC	
(ACS2)			
AT2G19590	ACCTCAGATGCAGATTGGGAAAGC	CCATCGTCTTGCTGAGTTCCTCTG	
(ACO1)			



Figure S1. Scheme illustrating the main steps of a phenotyping workflow to generate standardised output results.



Figure S2. Effect of waterlogging and submergence on plant size in separate, independent experiments. (a) Plant size (Projected Leaf Area) in aerobic plants (green box) compared to plants that were waterlogged (blue box). Data were taken at day 28. (b) Plant size (Projected Leaf Area) in aerobic plants (green box) compared to plants that were submerged (blue box). Data were taken at day 33. The experimental set-up is the same as in Figures 4 and 6.



Figure S3. GUS staining of *pADH1:GUS* plants in air or 5-days waterlogging in wo independent transgenic lines. The experimental set-up is the same as in Figure 7a.



Figure S4. Gel staining with Amido Black demonstrating equal loading in the SDS-PAGE utilized for the western-blot shown in Figures 7 and 8.



Figure S5. Activity of ADH in the samples utilized for the western-blot shown in Figures 7 and 8. One unit is the amount of enzyme leading to a change in A340 of 0.1 in one minute.



Figure S6. Role of ethylene in the induction of ADH1 and PDC1 in waterlogged plants. (a) Induction (RT-qPCR) of ACO1 and ACS2 by 5-days waterlogging; Statistically significant differences is indicated by the asterisk (T-test, pairwise comparison at each time point, *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.001). (b) Expression of ADH1 in aerobic (air) and 5-days waterlogged (WL) plants. The genotypes used are shown in figure. Different letters indicate statistically significant differences (two-way ANOVA). (c) Expression of PDC1 in aerobic (air) and waterlogged (WL) plants. The genotypes used are shown in figure. Different letters indicate statistically significant differences (ANOVA).



Figure S7. GUS staining of pADH1:GUS plants in air or 5-days waterlogging in wo independent transgenic lines. The experimental set-up is the same as in Figure 8a.



Figure S8. Role of ethylene in the induction of *ADH1* and *PDC1* in submerged plants. (a) Induction (RTqPCR) of *ACO1* and *ACS2* by 35h submergence; Statistically significant differences is indicated by the asterisk (T-test, pairwise comparison at each time point, *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001). (b) Expression of *ADH1* in aerobic (air) and 35h submerged (Sub) plants. The genotypes used are shown in figure. Different letters indicate statistically significant differences (twoway ANOVA). (c) Expression of *PDC1* in aerobic (air) and submerged (Sub) plants. The genotypes used are shown in figure. Different letter indicates statistically significant differences (ANOVA).



Figure S9. Description of the phenotypic parameters measured. (a) Projected Leaf Area: area of the rosette. (b) Compactness: Rosette Area/Conhull area. (c) Rosette Area / Area of min enclosing circle.