

Supporting Information tables

Article title: WAX INDUCER 1 regulates β -diketone biosynthesis by mediating expression of the *Cer-cqu* gene cluster in barley

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Table S1. General phenotyping of win1 KO lines as compared to the wild-type with standard deviations being indicated.

Trait	Control line	Win1 KO lines		
	WT	17-4-14	25-2-2	25-2-18
Spikelet number per spike	11.8 ± 0.6	12.8 ± 1.0	12.4 ± 0.8	12 ± 1.3
Spike length, cm	8.7 ± 0.9	8.7 ± 0.3	8.6 ± 0.9	8.8 ± 0.9
Grain number per spike	14 ± 4.6	18.6 ± 3.2	17.2 ± 3	16.8 ± 4
Grain number per plant	111.3 ± 33.8	139.9 ± 48.8	122.4 ± 24.3	100.3 ± 32.4
Grain weight per spike, g	0.66 ± 0.2	0.6 ± 0.24	0.78 ± 0.13	0.76 ± 0.17
Grain weight per plant, g	4.12 ± 0.81	4.64 ± 1.87	5.37 ± 1.36	4.44 ± 1.63
1000-grain weight, g	38.1 ± 5.4	32.7 ± 4.2	43.6 ± 4.7	43.5 ± 4.5
Plant height, cm	62.5 ± 4.6	65.1 ± 4.2	66.5 ± 3.1	64.8 ± 4.4
Total number of tillers	9.1 ± 2.7	9.9 ± 2.6	8.6 ± 1.8	7.4 ± 3.1
Number of fertile tillers	7.9 ± 2.3	8 ± 2.7	7.9 ± 1.8	6.6 ± 2.5

Table S2. Chemical composition of cuticular waxes detected on barley leaf blades and stems after derivatization.

			Content [µg/cm²]			
IUPAC Name	Linear retention index (LRI), exp.		Leaf blade		Stem	
			Control n=4	Mutant n=4	Control n=4	Mutant n=4
Hydrocarbons						
1.	Pentacosane	2500	0.029±0.009	0.018±0.003	0.05±0.01	0.025±0.008
2.	Hexacosane	2600	0.013±0.010	0.002±0.001	0.011±0.005	0.002±0.001
3.	Octacosane	2800	0.009±0.007	0.010±0.002	0.011±0.001	0.008±0.004
4.	Nonacosane	2900	a	a	0.044±0.006	0.052±0.010
5.	Triacontane	3100	0.010±0.004	0.006±0.004	0.017±0.005	0.008±0.003
6.	Trtriacontane	3300	0.021±0.008	0.020±0.005	0.010±0.003	0.006±0.002
Saturated fatty acids methyl esters						
7.	Hexadecanoic acid	1928	0.34±0.12	0.19±0.04	0.33±0.08	0.16±0.02
8.	Octadecanoic acid	2128	0.17±0.06	0.12±0.02	0.35±0.07	0.07±0.01
9.	Eicosanoic acid	2329	0.25±0.04	0.28±0.05	0.93±0.16	0.14±0.01
10.	Docosanoic acid	2532	0.37±0.07	0.43±0.07	0.40±0.10	0.30±0.06
11.	Tetracosanoic acid	2731	0.19±0.03	0.21±0.02	0.16±10.04	0.08±0.02
12.	Hexacosanoic acid	2936	0.37±0.09	0.46±0.08	0.08±0.01	0.10±0.02
13.	Octacosanoic acid	3138	0.19±0.03	0.19±0.05	0.06±0.02	0.07±0.02
14.	Triacontanoic acid	3342	0.11±0.02	0.11±0.02	0.04±0.01	0.05±0.02
15.	Dotriacontanoic acid	3542	0.06±0.01	0.06±0.01	0.07±0.02	0.03±0.01
16.	Tetratriacontanoic acid	3741	0.04±0.01	0.03±0.01	0.04±0.01	0.02±0.01
Unsaturated fatty acids methyl esters						
17.	Octadecenoic acid	2098	0.06±0.02	0.02±0.01	0.22±0.07	0.04±0.02
18.	Octadecadienoic acid	2092	0.03±0.01	0.01±0.01	0.04±0.01	0.03±0.01
19.	Octadecatrienoic acid	2098	0.06±0.02	0.02±0.01	0.07±0.04	0.04±0.01
Aldehydes						
20.	Hexacosanal	2833	0.72±0.15	0.49±0.03	0.16±0.04	0.10±0.03
21.	Octacosanal	3043	0.72±0.18	0.54±0.10	0.12±0.05	0.08±0.02
22.	Triacontanal	3244	0.04±0.01	<0.01	0.023±0.006	0.036±0.014
23.	Dotriacontanal	3452	<0.01	<0.01	0.18±0.09	0.02±0.01
24.	Tetratriacontanal	3652	<0.01	<0.01	0.10±0.04	0.007±0.005
Alkan-2-ols						
25.	Tridecan-2-ol	1503	b	b	0.16±0.05	0.002±0.001

26.	Pentadecan-2-ol	1704	b	b	0.22±0.05	0.007±0.001
<i>Alkan-1-ols</i>						
27.	Docosan-1-ol	2489	0.44±0.11	0.34±0.07	0.18±0.04	0.33±0.07
28.	Tetracosan-1-ol	2694	0.63±0.09	0.34±0.07	0.12±0.06	0.35±0.07
29.	Hexacosan-1-ol	2901	10.8±1.1	7.7±0.7	1.1±0.2	1.6±0.3
30.	Octacosan-1-ol	3103	0.51±0.09	0.43±0.04	b	0.18±0.05
31.	Triacontan-1-ol	3309	0.12±0.02	0.09±0.02	b	0.03±0.01
<i>β-diketones</i>						
32.	Hentriacontane-14,16-dione	3395	b	b	29.7±2.8	1.7±0.5
33.	25-Hydroxy-hentriacontane-14,16-dione	3586	b	b	1.7±0.5	b
<i>Alkylresorcinols</i>						
34.	3-Methoxy-5-pentadecylphenol	2718	b	b	a	a
35.	3-Methoxy-5-heptadecylphenol	2935	b	b	0.36±0.12	0.04±0.01
36.	3-Methoxy-5-nonadecylphenol	3144	b	b	0.20±0.08	0.007±0.004
37.	3-Methoxy-5-heneicosylphenol	3351	b	b	c	c
38.	2-Methyl-5-nonadecyl-1,3-benzenediol	3268	0.04±0.01	0.05±0.03	b	0.06±0.02
39.	2-Methyl-5-heneicosyl-1,3-benzenediol	3481	0.05±0.02	0.13±0.07	b	0.03±0.01
40.	2-Methyl-5-tricosyl-1,3-benzenediol	3687	b	0.02±0.01	b	0.01±0.01

^a it was impossible to determine the individual contents due to overlapping peaks of the corresponding alcohols, ^b not found, ^c was identified below the limit of determination

Table S3. Differential expression of all barley genes in all four performed comparisons. Sheets:

lb_win1-lb_wt: comparison between leaf blade of *win1* knockout genotype and leaf blade of wild type plants; ls_win1-ls_wt: comparison between leaf sheath of *win1* knockout genotype and leaf sheath of wild-type plants; lb_win1-ls_win1: comparison between leaf blade of *win1* knockout plants and leaf sheath of *win1* knockout plants; lb_wt-ls_wt: comparison between leaf blade and leaf sheath of wild-type plants.

Table S4. Information about the *Cer-cqu* cluster and related genes located on chromosome 2H.

Table S5. Barley homologues of *Arabidopsis thaliana* genes involved in wax biosynthesis.**Table S6.** Expression (rpkm-normalized counts) of putative WIN1-regulated genes.**Table S7.** Hexamers enriched in promoters of WIN1-related genes. Sheets: leaf sheath wt upreg – hexamers enriched in promoters of genes up-regulated in wild-type leaf sheaths, leaf sheath wt downreg – hexamers enriched in promoters of genes down-regulated in wild-type leaf sheaths.**Table S7.** Primers used in the study.

Gene id	Primer	Sequence	Amplicon length [b]	Optimal annealing temperature [°C]
Primers used for target loci sequencing				
HORVU6Hr1G038120 (<i>HvWIN1</i>)	Win1_F	CGTACTAAGTGACTCCAGCAG	450	60
	Win1_R	TAAACCGGCGAAGAAACCAC		
HORVU6Hr1G085850	6H_gene_F	GAGAAGTCCGAGTCTAGCAG	960	59
	6H_gene_R	AAACGGGCTAAACAAAGATGTC		
HORVU7Hr1G029870	7H_gene_F	TTTGCCAACTAAACGTGTCACC	530	62
	7H_gene_R	CAATCGCAGAGCCTTTCGAC		
HORVU7Hr1G089930 (<i>HvNUD</i>)	Hv_Nud_1exF	AGCGTAGGTGTATACGCATCCG	1000	58
	Hv_Nud_R2	CAATGTGGGACTTCTCGGTG		
Primers used for qRT-PCR				
HORVU2Hr1G002570	2570_F1	AGACGACCTGCTTGGGCAAATG	116	60
	2570_R1	CGTAACCAGCCGCGAAGAAAG		
HORVU2Hr1G002580	2580_F1	CGGGTTGCGGTTCTTTGTCAC	174	62.5
	2580_R1	GCCCTTTACTTTTGCGCGCTG		
HORVU2Hr1G002610	2610_F1	GGCCCCATAGCTTTCCTGCA	74	60

HORVU2Hr1G002620	2610_R1	G TTCCTTTCGGCACCTTCACG	117	60
	2620_F1	TATCCCGTAGGTACCCGTCA		
	2620_R1	GGCCATCCTTGTACTTGCGG		
HORVU2Hr1G002630	2630_F1	TTGCCGGCGTGTGGTTTACG	129	60
	2630_R1	CATGGTAGTGTGCGTAAGCGC		
HORVU2Hr1G002640	2640_F1	TCCGTCTCTCAAACCCTCGTG	102	60
	2640_R1	CAGTGTGGGCAGTTTGGTGTA		
HORVU2Hr1G002650	2650_F1	GTGAAGGTGCCGAAAGGAACG	135	60
	2650_R1	GACACAGACCCTCGGCCC		
HORVU3Hr1G085520	5520_F1	CGCTCAACACGCACCTCTTC	89	60
	5520_R1	CGTGGTGTAGTTGGCGGTGTA		
HORVU3Hr1G086160	6160_F3	GGCCCCATCGTCATCCTCC	100	61
	6160_R3	CCTCCGTGTCCGCCTTCC		
HORVU3Hr1G086200	6200_F4	CCGCAGCCTCGCCACAAAAC	77	62
	6200_R4	TCTTGGGGCAGGGGAGCG		
HORVU1Hr1G087320	7320_F1	GCTCACGCTCGACCAGGG	147	60
	7320_R1	TCGCCCTCCCAGACGTC		
HORVU6Hr1G075900	5900_F1	CGGGAGGCGTTCGTGGAG	124	60
	5900_R1	TGGTGTGACGGCGAGCTTG		
HORVU3Hr1G077960	7960_F1	GGCGCTGGTGCTGGATCAAAG	65	60
	7960_R1	CTCTTTCTTCCGTTGCGCTGC		
HORVU2Hr1G032710	2710_F1	AGCCAGCAAGAACCACCGAGAA	165	60
	2710_R1	CAGACTTGATTGAGCCCTTGC		
HORVU7Hr1G098280	8280_F1	TCCGGGTTCCAGTCCAAGCA	70	60
	8280_R1	GCCGGGGACGAGCTTGAG		
HORVU5Hr1G046520	6520_F1	ACCACCACCCACTCATCTG	162	60
	6520_R1	GAGTCCACCTGCCCGCAC		
Primers used for transgene analysis				
	Cas9_F	GACGATGACCTGGATAACCT	300	58
	Cas9_R	GATGAACTTGTAAGAACTCCTCT		58

Table S8. Counts per million mapped read (CPM)-normalized counts of gene expression, obtained using the edgeR software package, raw data. lb_win1, lb_wt – leaf blade wild-type and *win1* KO respectively; ls_wt, ls_win1 – leaf sheath wild-type and *win1* KO respectively. Numbers represent biological replicates.

Table S9. Raw counts of gene expression obtained using DART mapper and featureCounts function of the Subread package. lb_win1, lb_wt – leaf blade wild-type and *win1* KO respectively; ls_wt, ls_win1 – leaf sheath wild-type and *win1* KO respectively. Numbers represent biological replicates.