

Figure S1 Transcript length distribution of the 131,457 transcripts included in the RTA


Figure S2 PCA plot of 397 samples with more than 0.5 million mapped reads based on the 500 transcripts with highest variance.


Figure S3 Distribution of Pearson correlation coefficients of biological replicates from Greenhouse samples, Field samples and among samples across the two sites

(C)

$-\log 10(F D R)$
25
20
15
10
5
0

Figure S4 Biological process (A), cellular compartments (B) and molecular function (C) GO terms enriched for differentially expressed transcript sets between adjacent time points. FDR adjusted p-values < 0.01 (in -log10 scale) were colored between blue and red, and cells without GO terms assigned were colored in gray.


Figure S5 The 80 observed temporal transcript expression patterns identified from 25,971 differentially expressed transcripts between five pairs of adjacent time points


Figure S6 Correlation of transcript numbers (log scale) between each pair of symmetrical upand down-regulated expression patterns. Each point represents a pair of symmetrical up- and down-regulated expression patterns. The number of transcripts in the up-regulated pattern on the $x$-axis and the number of transcript in the down-regulated pattern on the $y$-axis. Black points have one differential expression event, red points two, and green points three such events.



Figure S8 Distribution of $P$ values of simple linear regression between 634 metabolites and PC1 scores of GCoE sets. Red boxes contained p-values from real data, and blue boxes contained p-values from 100 permutations.


Figure S9 Transcript length distribution of the 9,817 transcripts that couldn't be aligned to the UniRef100


Figure S10 clusters of 12 HiSeq samples based on expression profiles. Euclidean distances between samples were colored between dark blue and light blue.


Figure S11 Pearson correlation coefficients of biological replicates from 12 HiSeq samples of cv.Ogle-C whose developing seeds were collected at 7, 14, 21, and 28 DAA


Figure S12 Heatmap of genomic relationship among 22 oat lines used in this study

52 Table S1 A comparison of BUSCOs plant gene completeness between the RTA in this study and the first version of de novo oat seed transcriptome assembly (dnOST, Gutierrez-Gonzalez et al.
54 2013)

| BUSCO Statistics | the RTA | $d n$ OST |
| :--- | :--- | :--- |
| Complete BUSCOs | $1212(84.2 \%)$ | $412(28.7 \%)$ |
| Complete and single-copy BUSCOs | $1188(82.5 \%)$ | $116(8.1 \%)$ |
| Complete and duplicated BUSCOs | $24(1.7 \%)$ | $296(20.6 \%)$ |
| Fragmented BUSCOs | $148(10.3 \%)$ | $201(14.0 \%)$ |
| Missing BUSCOs | $80(5.5 \%)$ | $827(57.3 \%)$ |
| Total BUSCO groups searched | 1440 | 1440 |

57 Table S2 Chi-Square test for sub-cluster size distribution of the 22 temporal co-expression sets.
58 At significant level of 0.05 , the critical p-value after Bonferroni correction is $0.05 / 22=0.00227$.
59

| Temporal co-expression set* P-value |  |
| :--- | :--- |
| Top-8 | 0.994548451 |
| Top-10 | 0.999286221 |
| Top-12 | 0.497109215 |
| Top-15 | 0.003939038 |
| Top-13 | 0.108831006 |
| Top-11 | 0.344207141 |
| Top-9 | 0.380203442 |
| Top-7 | 0.608797221 |
| Top-3 | 0.895528366 |
| Top-6 | 0.419936316 |
| Top-5 | 0.273601551 |
| Top-4 | 0.594794097 |
| Top-31 | 0.021221027 |
| Top-16 | 0.133281338 |
| Top-1 | 0.637929437 |
| Top-2 | 0.83840654 |
| Top-24 | 0.744979403 |
| Top-18 | 0.210760149 |
| Top-19 | 0.980442768 |
| Top-20 | 0.35296887 |
| Top-14 | 0.962213032 |
| Top-17 | 0.549011073 |

60 *The temporal co-expression sets were ordered the same as in Figure 4.

Table S3 A list of oat transcripts homologous to biosynthetic genes of avenanthremides and fatty acids from other oat cultivars and Brachypodium distachyon

| Genename | Source | Transcript name | reference | query_len ${ }^{\text {a }}$ | ref_len ${ }^{\text {b }}$ | aln_length ${ }^{\text {c }}$ | pct_ident ${ }^{\text {d }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Avenanthramides biosynthetic genes |  |  |  |  |  |  |  |
| CCoA3H | Arabidopsis | TRINITY_DN6473_c0_g1_i1 | NM_180006.2 | 1942 | 1885 | 1362 | 67.621 |
| CCoA3H | Brachypodium | TRINITY_DN6473_c0_g1_i1 | KQK05634 | 1942 | 2031 | 1954 | 86.131 |
| CCoAOMT | Oat | TRINITY_DN3508_c0_g1_i7 | AB076979.1 | 1238 | 622 | 604 | 99.834 |
| HHT1 | Oat | TRINITY_DN5172_c0_g1_i4 | AB076980.1 | 1598 | 1755 | 1343 | 79.449 |
| HHT2 | Oat | TRINITY_DN5172_c0_g1_i4 | AB076981.1 | 1598 | 1770 | 1343 | 78.555 |
| HHT3 | Oat | TRINITY_DN5172_c0_g1_i4 | AB076982.1 | 1598 | 1659 | 1343 | 79.598 |
| HHT4 | Oat | TRINITY_DN5172_c0_g1_i4 | AB076983.1 | 1598 | 1100 | 1088 | 97.151 |
| Fatty Acids biosynthetic genes |  |  |  |  |  |  |  |
| ACCase | Brachypodium | TRINITY_DN146_c0_g1_i8 | BRADI_5g03860 | 7812 | 8783 | 7598 | 88.694 |
| DGAT1/TAG1 | Brachypodium | TRINITY_DN151_c0_g1_i4 | BRADI_1g37750 | 2878 | 3009 | 925 | 72.757 |
| FAB1/KAS2 | Brachypodium | TRINITY_DN207_c0_g1_i1 | BRADI_1g60300 | 2876 | 2498 | 1851 | 87.088 |
| FAB2 | Brachypodium | TRINITY_DN2740_c0_g1_i5 | BRADI_2g58930 | 5078 | 1792 | 1610 | 86.957 |
| FAD2 | Brachypodium | TRINITY_DN268_c0_g1_i4 | BRADI_3g53370 | 1883 | 2351 | 1665 | 85.526 |
| FAD3 | Brachypodium | TRINITY_DN10818_c0_g1_i3 | BRADI_1g65580 | 1198 | 2817 | 1195 | 82.343 |
| FAE1/KCS18 | Brachypodium | TRINITY_DN1011_c0_g1_i5 | BRADI_2g16050 | 1990 | 2481 | 1447 | 81.064 |
| FATB | Brachypodium | TRINITY_DN2629_c0_g1_i1 | BRADI_1g51170 | 2394 | 4407 | 1368 | 88.085 |
| GPAT9 | Brachypodium | TRINITY_DN5048_c0_g1_i7 | BRADI_1g25790 | 1490 | 1540 | 1460 | 86.37 |
| LPCAT1 | Brachypodium | TRINITY_DN3352_c0_g1_i1 | BRADI_3g51577 | 1846 | 1671 | 1634 | 88.433 |
| PAH1 | Brachypodium | TRINITY_DN1042_c0_g1_i6 | BRADI_2g23040 | 4252 | 4162 | 2485 | 82.817 |
| PDAT1 | Brachypodium | TRINITY_DN2921_c0_g1_i1 | BRADI_4g31540 | 2494 | 2601 | 2531 | 86.448 |
| WRI1 | Brachypodium | TRINITY_DN331_c0_g1_i1 | BRADI_4g43877 | 3900 | 2035 | 1223 | 80.376 |

63 aquery_len= query sequence length;
64 bref_len = reference sequence length;
65 caln_length= alignment length;
66 dpct_ident= percent identity

