Table S1. Gene primers for qRT-PCR validation

|  |  |  |  |
| --- | --- | --- | --- |
| GENE | primer name |  | sequence |
| comp59407\_c0 | comp59407\_c0-R | 5’ primer | TCATCCCCACCAAGAAGTCACA |
|  | comp59407\_c0-F | 3’ primer | GCCATTAAGGTCGAACTTTCCG |
| comp58684\_c0 | comp58684\_c0-F | 5’ primer | ATCACGCCCATCGTCATCAT |
|  | comp58684\_c0-R | 3’ primer | CGGGAAGAACTTCTCCAGAAAA |
| comp58560\_c1 | comp58560\_c1-1-F | 5’ primer | AGGGATGAATCGTGGGTATGC |
|  | comp58560\_c1-1-R | 3’ primer | GGGTAGGAATCAGCGAAGGAA |
| comp58741\_c0 | comp58741\_c0-1-F | 5’ primer | ACAACGCAGGTCTAAAGCTCAG |
|  | comp58741\_c0-1-R | 3’ primer | GACAGAAGTAACACCACGCACA |
| comp52868\_c0 | comp52868\_c0-1-F | 5’ primer | AAACAGGTGGTCCAGGATAGC |
|  | comp52868\_c0-1-R | 3’ primer | TGAAGATGAAGATGACGAGGGT |
| comp52741\_c0 | comp52741\_c0-1-F | 5’ primer | ATGATCCGTCCACTCGTTGT |
|  | comp52741\_c0-1-R | 3’ primer | CATGCTGGCCTTCTCGTAAC |

Table S2. Overview of RNA sequencing result

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Raw Data |  | Valid Data |  | Valid% | Q20% | Q30% | GC% | Expressed gene number |
|  | Read | Base | Read | Base |  |  |  |  |  |
| CK\_L1\_CK\_L | 59056696 | 8.92G | 57065926 | 8.11G | 96.63 | 96.25 | 90.53 | 57.97 | 36787 |
| CK\_L2\_CK\_L | 43200284 | 6.52G | 41922812 | 6.03G | 97.04 | 97.03 | 91.82 | 57.43 | 36675 |
| CK\_R1\_CK\_R | 41099034 | 6.21G | 39939640 | 5.74G | 97.18 | 97.15 | 92.16 | 56.89 | 42652 |
| CK\_R2\_CK\_R | 45802174 | 6.92G | 44438906 | 6.42G | 97.02 | 97.23 | 92.42 | 54.90 | 42033 |
| D18d\_L1\_D18d\_L | 46438216 | 7.01G | 44694278 | 6.38G | 96.24 | 96.61 | 90.87 | 57.35 | 37114 |
| D18d\_L2\_D18d\_L | 40699350 | 6.15G | 39401744 | 5.65G | 96.81 | 96.84 | 91.56 | 57.29 | 37099 |
| D18d\_R1\_D18d\_R | 68273904 | 10.31G | 66348806 | 9.60G | 97.18 | 97.26 | 92.60 | 56.61 | 41075 |
| D18d\_R2\_D18d\_R | 41667486 | 6.29G | 40320554 | 5.78G | 96.77 | 96.92 | 91.59 | 57.43 | 37491 |

Table S3. Overview of RNA sequencing reads from raw data to cleaned sequences.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | CK\_R\_1 |  | CK\_R\_2 |  | CK\_L\_1 |  | CK\_L\_2 |  | D18d\_L\_1 |  | D18d\_L\_2 |  | D18d\_R\_1 |  | D18d\_R\_2 |  |
| lib | type | Total | uniq | Total | uniq | Total | uniq | Total | uniq | Total | uniq | Total | uniq | Total | uniq | Total | uniq |
| Raw reads | NA | 11858207 | 2691231 | 11254649 | 3779749 | 17825489 | 2501656 | 13249071 | 2611194 | 10170218 | 1138572 | 16615428 | 3144401 | 18715594 | 3125474 | 15237544 | 3587407 |
| 3ADT&length filter | Sequence type | 4632909 | 1241316 | 4784181 | 1526674 | 9511873 | 1456316 | 7197982 | 970906 | 3287076 | 493255 | 13592051 | 2047265 | 8039114 | 1786508 | 5573741 | 1813205 |
| Junk reads | Sequence type | 45669 | 21791 | 71678 | 31009 | 30736 | 16211 | 64622 | 26315 | 24118 | 9700 | 21872 | 9869 | 58330 | 16288 | 60103 | 19230 |
| Rfam | RNA class | 1379134 | 76468 | 1172241 | 128371 | 1879221 | 73586 | 490011 | 45176 | 1332710 | 42848 | 285918 | 30920 | 1810045 | 132362 | 2045108 | 192651 |
| Repeats | RNA class | 2427 | 1271 | 12778 | 5536 | 2622 | 1392 | 7955 | 1874 | 3265 | 918 | 6458 | 3367 | 3186 | 1707 | 21084 | 4748 |
| valid reads | Sequence type | 5798999 | 1350684 | 5217778 | 2089353 | 6401823 | 954628 | 5490937 | 1567367 | 5524004 | 592030 | 2710475 | 1053448 | 8805921 | 1189121 | 7547616 | 1558866 |
| rRNA | RNA class | 755625 | 44925 | 692062 | 65467 | 1272555 | 46824 | 242151 | 24478 | 917663 | 25846 | 88867 | 14573 | 1405527 | 91153 | 1417561 | 114648 |
| tRNA | RNA class | 436388 | 17355 | 269049 | 35316 | 485623 | 15824 | 201925 | 11636 | 330974 | 10137 | 174066 | 8051 | 222866 | 25435 | 389723 | 52157 |
| snoRNA | RNA class | 38625 | 3471 | 59440 | 7570 | 32131 | 2371 | 18638 | 2800 | 12780 | 1456 | 9011 | 2482 | 21171 | 2884 | 51445 | 5782 |
| snRNA | RNA class | 73097 | 5240 | 57929 | 9172 | 12328 | 3160 | 5001 | 2266 | 12457 | 1819 | 3162 | 1868 | 71941 | 5082 | 66751 | 7286 |
| other Rfam RNA | RNA class | 75399 | 5477 | 93761 | 10846 | 76584 | 5407 | 22296 | 3996 | 58836 | 3590 | 10812 | 3946 | 88540 | 7808 | 119628 | 12778 |

Note: 3ADT&length filter: reads removed due to 3ADT not found and length with <18 nt and >25 nt were removed(for plants); length with<18 and >26 were remove(for animals)

Junk reads:Junk: >=2N, >=7A, >=8C, >=6G, >=7T, >=10Dimer, >=6Trimer, or >=5Tetramer

Rfam: Collection of many common non-coding RNA families except micro RNA; http://rfam.janelia.org

Repeats: Prototypic sequences representing repetitive DNA from different eukaryotic species; http://www.girinst.org/repbase.

Table S4. All expressed miRNA detected by miRNA sequencing. (attached with an excel file)

Table S5. Length distribution of unique miRNAs

|  |  |  |
| --- | --- | --- |
| Length | Unique miRNA | % |
| 18 | 108 | 17.76 |
| 19 | 89 | 14.64 |
| 20 | 72 | 11.84 |
| 21 | 215 | 35.36 |
| 22 | 57 | 9.38 |
| 23 | 14 | 2.30 |
| 24 | 47 | 7.73 |
| 25 | 6 | 0.99 |
| all | 608 | 100.00 |

Table S6. miRNA targets by TargetFinder software (attached with an excel file)

Table S7 Degradome-seq overview of reads from raw data to mapping sequences

|  |  |  |
| --- | --- | --- |
| Sample | number | ratio |
| Raw Reads | 35065665 | / |
| reads < 15nt after removing 3 adaptor | 140807 | 0.40% |
| Mappable Reads | 34924858 | 99.60% |
| Unique Raw Reads | 9875364 | / |
| Unique reads < 15nt after removing 3 adaptor | 42925 | 0.43% |
| Unique Mappable Reads | 9832439 | 99.57% |
| Transcript Mapped Reads | 21363848 | 60.93% |
| Unique Transcript Mapped Reads | 4864003 | 49.25% |
| Number of input Transcript | 55366 | / |
| Number of Coverd Transcript | 45765 | 82.66% |

Table S8. Degradome result (attached with an excel file)

Table S9. GO annotation of miRNA and targeted genes (attached with an excel file)

Table S10. 41 differently expressed miRNAs in different comparisons

|  |  |  |  |
| --- | --- | --- | --- |
| D18LvsD18R | CKLvsCKR | D18LvsCKL | D18RvsCKR |
| ata-miR164c-3p | bdi-miR160e-5p\_L+1R-1 | bdi-MIR7757-p5\_1ss4TC | bdi-MIR1135-p3\_2ss17CT20GT |
| bdi-miR162\_R+1 | bdi-miR166b-5p | osa-miR444a-3p.2 | sbi-MIR396d-p3\_2ss2CA18TC |
| bdi-miR528-5p | ata-miR393-3p\_L+1R-3 | osa-MIR812q-p3\_2ss10GA20TG | far-MIR1122-p5\_1ss9AG |
| bdi-miR528-5p\_R-3\_1ss18GT | sbi-MIR396d-p3\_2ss2CA18TC | bdi-MIR5174c-p5\_2ss5TC17TA | bdi-miR5049-5p\_R-2\_2ss4TC20TC |
| sbi-miR171e\_L-1R+2 | PC-3p-68901\_67 | tae-MIR9775-p3\_1ss11TC\_3 | ata-miR164c-3p |
| tae-MIR9775-p3\_2ss7TA19AT\_2 | ata-miR164c-3p | tae-MIR9775-p3\_1ss11TC\_2 | zma-miR162-5p |
| tae-MIR9775-p5\_2ss7TA19AT\_2 | far-MIR1122-p5\_1ss9AG | bdi-MIR7757-p3\_2ss5TC17TC | bdi-MIR7757-p5\_1ss3AC |
| tae-MIR9775-p3\_2ss7TA19AT\_1 | bdi-miR166a-3p\_L+2R-2 | tae-miR1130b-3p\_L+4R-6\_1ss6CT |  |
| tae-MIR9775-p5\_2ss7TA19AT\_1 | osa-MIR812q-p3\_2ss10GA20TG | far-MIR159-p3\_2ss18TA19CT |  |
| bdi-miR5049-3p\_L-4R+1\_1ss21AG | tae-miR1130b-3p\_L+4R-6\_1ss6CT |  |  |
| ata-miR393-3p\_L+1R-3 | tae-miR319\_L-1R+1 |  |  |
| osa-miR444a-3p.2\_L-3 | tae-MIR7757-p5\_1ss11TG |  |  |
| bdi-MIR7757-p3\_2ss1CA18CT |  |  |  |
| bdi-miR156f-3p\_2ss8GT13AC |  |  |  |
| PC-3p-68901\_67 |  |  |  |
| tae-miR5049-3p\_L-1\_1ss8AG |  |  |  |
| bdi-MIR5174c-p5\_2ss5TC17TA |  |  |  |
| tae-MIR1120b-p5\_2ss9TC20AG |  |  |  |
| tae-MIR1122a-p5\_1ss9GA |  |  |  |
| tae-MIR9775-p3\_1ss11TC\_3 |  |  |  |
| tae-MIR9775-p3\_1ss11TC\_2 |  |  |  |
| tae-miR1133\_L-1R+4\_1ss19GC |  |  |  |
| PC-5p-1284015\_3 |  |  |  |
| osa-miR6250\_L-1R+2\_1ss14CT |  |  |  |
| bdi-MIR1135-p3\_2ss17CT20GT |  |  |  |
| osa-miR444a-3p.2 |  |  |  |



Figure S1. GO and KEGG annotation of 378 common DEGs in leaf and root



Figure S2. Length distribution of counts of total and unique sRNAs



Figure S3. Degradome KEGG significant scatterplot