

**Article title:** Accumulation of miRNAs differentially modulated by drought is affected by grafting in grapevine

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## Supplementary material

**Table S1.** Stem loop primers used in this study for miRNA-specific RT-PCR assay. The last eight bases at 3' of each primer are highlighted to indicate the region complementary to the miRNA sequence.

| Primer description          | Type of miRNA | Stem-loop primer sequence (5'-3')                       |
|-----------------------------|---------------|---|
| <i>Stem Vvi-miR156b,c,d</i> | conserved     | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGGTGCTCAC        |
| <i>Stem Vvi-miR159c</i>     | conserved     | CGCAGTTACTTCGAGAGAGAGAGAGAGACGAAGTAAC TGCGTAGAGCTC      |
| <i>Stem Vvi-miR393a</i>     | conserved     | CGCAGTTACTTCGAGAGAGAGAGAGAGACGAAGTAAC TGCGGATCAATG      |
| <i>Stem Vvi-miR396c,d</i>   | conserved     | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGCAGTCAA         |
| <i>Stem Vvi-miR3624</i>     | known         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGAGTAGTAT        |
| <i>Stem n_22</i>            | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACAATGAAA*  |
| <i>Stem n_47</i>            | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGGGATGGG         |
| <i>Stem n_231</i>           | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACGGAGGAAT* |
| <i>Stem n_479</i>           | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGGGATGGG         |
| <i>Stem n_520</i>           | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACGATGACAG* |
| <i>Stem n_622</i>           | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACGCTGACAG* |
| <i>Stem n_55</i>            | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGCTATCAAT        |
| <i>Stem n_191</i>           | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGTGCAGGTG        |
| <i>Stem n_312</i>           | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACCATGTGAT* |
| <i>Stem n_327</i>           | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGTGTGCTCT        |
| <i>Stem n_337</i>           | novel         | CGCAGTTACTTCGAGAGAGAGAGAGACGAAGTAAC TGCGTCTTATCC        |
| <i>Stem n_346</i>           | novel         | GTCGTATCCAGTGCAGGGTCCGAGGTATT CGCACTGGATA CGACGGTCCCC*  |

\* from Chen et al. (2005)

**Table S2.** Oligonucleotides used in this study for RT-qPCR analysis of miRNAs and related target transcripts.

| Gene description         | Gene ID<br>(miRBase V.22<br>or VVGDB 12X<br>V1) | Primer                 | Primer sequence (5'-3') | Product size<br>(bp) |
|--------------------------|---|------------------------|-------------------------|----------------------|
| <i>Vvi-miR156b,c,d</i>   | MI0006486-<br>MI0006487-<br>MI0006488           | Forward                | ACAGAAGAGAGTGAGCACCG    | 61                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>Vvi-miR159c</i>       | MI0006495                                       | Forward                | GGATTGAAGGGAGCTCTACG    | 62                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>Vvi-miR393a</i>       | MI0007952                                       | Forward                | CCAAAGGGATCGCATTG       | 61                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>Vvi-miR396c,d</i>     | MI0007955-<br>MI0006571                         | Forward                | TTCCACAGCTTCTTGAAC TG   | 62                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>Vvi-miR3624</i>       | MI0016017                                       | Forward                | TCAGGGCAGCAGCATACTAC    | 62                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 22 (n_22)</i>   |   | Forward                | GCGTAGACCATGCATTTCA     | 64                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |
| <i>novel 47 (n_47)</i>   |   | Forward                | TTCCAATGCCGCCCA         | 63                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 231 (n_231)</i> |   | Forward                | GCCAAGAACGACATT CCTCC   | 65                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |
| <i>novel 479 (n_479)</i> |   | Forward                | TCTTACCAACACCTCCCATTCC  | 63                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 520 (n_520)</i> |   | Forward                | GCCGGCTCTCTAGACTTCTGT   | 65                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |
| <i>novel 622 (n_622)</i> |   | Forward                | CGCTCATTTCTCTTCTGTCA    | 66                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |
| <i>novel 55 (n_55)</i>   |   | Forward                | ATTCTTATCGATTGATAGCGCAG | 59                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 191 (n_191)</i> |   | Forward                | GACTTAGGAAGAACATGCACC   | 64                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 312 (n_312)</i> |   | Forward                | GGAGTGACCTGAGAACATCA    | 66                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |
| <i>novel 327 (n_327)</i> |   | Forward                | TTGACAAAGAGAGAGAGCACAC  | 62                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 337 (n_337)</i> |   | Forward                | TGTTGGTTAGTGATGGGATAAG  | 65                   |
|                          |   | Reverse A <sup>a</sup> | CGCAGTTACTTCGAGAGAGAGA  |                      |
| <i>novel 346 (n_346)</i> |   | Forward                | CTGGAAGCCGATGGGG        | 65                   |
|                          |   | Reverse B <sup>b</sup> | GTCGTATCCAGTGCAGGGT     |                      |

<sup>a,b</sup> Reverse A and B primers were the universal reverse primers respectively used for amplifying cDNA samples obtained using either the stem loop primer designed in this study or the stem loop primer by Chen et al. (2005) (see Table S1).

**Table S2.** Continued.

| Gene description  | Gene ID (miRBase V.22 or VVGDB 12X V1)   | Primer  | Primer sequence (5'-3') | Product size (bp) |
|---|--|---------|-------------------------|-------------------|
| <i>Metal ion binding protein (VvMetallothionein)</i>      | VIT_00s0194g00340                        | Forward | GCTGAGGGTTGATCTTGAGTG   | 92                |
|   |  | Reverse | CAAAGAAAGCCTGGTCTCGT    |                   |
| <i>Growth Regulating Factor-like 1 (VvGRL1-like)</i>      | VIT_00s0494g00010                        | Forward | CTATCAACCCACACCTCTGC    | 97                |
|   |  | Reverse | CCCTCTAACCCCCAGCAAAA    |                   |
| <i>Auxin signalling f-box 2 (VvAUXIN F-BOX 2)</i>         | VIT_14s0068g01330                        | Forward | CAGGTTGAAGAGAATGGTGGT   | 104               |
|   |  | Reverse | TGGTAACCCCTTCACAGCTG    |                   |
| <i>Constans-like protein 5 (VvCONSTANS-like)</i>          | VIT_04s0008g07340                        | Forward | GTCGATGGATCCAAAGCTTC    | 100               |
|   |  | Reverse | CACCAATGGAGCTTGAACACT   |                   |
| <i>Topless related protein (VvTOPLESS-like)</i>           | VIT_09s0002g08370                        | Forward | TGCAGTTCCCTCACCTCAAA    | 99                |
|   |  | Reverse | CTGGATTGGCCTTGGATT      |                   |
| <i>GATA transcription factor 26-like (VvGATA26-like)</i>  | VIT_00s0287g00040                        | Forward | GAAGGGTGATTATGAAGGCAG   | 104               |
|   |  | Reverse | AAGAGAGGCCACCATCAGGA    |                   |
| <i>MYB transcription factor (VvMYB-like)</i>              | VIT_19s0090g00590                        | Forward | TCTCTATCCCTCTCGACCC     | 96                |
|   |  | Reverse | GTCACCCGATCGCTTGTA      |                   |
| <i>Calmodulin binding protein MLO 16 (VvMLO16)</i>        | VIT_12s0035g00260                        | Forward | CCATTCATCTCTTCAACAGC    | 118               |
|   |  | Reverse | TTTGGGGGAATGTTAGTGTGTC  |                   |
| <i>Disease resistance protein NBS-LRR (VvNBS-LRR)</i>     | VIT_09s0096g00830                        | Forward | GGATGATCGGCTTATATGGGT   | 107               |
|   |  | Reverse | GGCCCAAATTACGACATCA     |                   |
| <i>Polyphenol oxidase, chloroplastic-like (VvPolyoxC)</i> | VIT_00s0480g00100                        | Forward | CATTGCGCCTATTGCCA       | 98                |
|   |  | Reverse | CACGTGTTGTATTGGTTGGC    |                   |
| <i>NBS-LRR defence protein RPS5-like (VvRPS5)</i>         | VIT_09s0002g04950                        | Forward | GGGTGGGAATTATGGTATTGTA  | 99                |
|   |  | Reverse | CTCAAAGTCATTGGATGTTGC   |                   |
| <i>Zinc Finger protein (VvZinc)</i>                       | VIT_06s0004g06440                        | Forward | GGGGAAGAGAAAGCCATCAT    | 100               |
|   |  | Reverse | GGTTCAATTCCCTCTCCTCTG   |                   |
| <i>Hexose transporter HT5 (VvHT5)</i>                     | VIT_05s0020g03140                        | Forward | CCCTGTTGTTAATGGAAGTGAC  | 92                |
|   |  | Reverse | CCTCCCCATACACCACCAATC   |                   |
| <i>CBL interacting protein kinase 13 (VvCIPK13)</i>       | VIT_13s0067g02480                        | Forward | GATAAGGCCAGCACTCAAGG    | 101               |
|   |  | Reverse | CCTGCAGTTCTGATATGGTTC   |                   |
| <i>Squamosa Promoter binding protein-Like 6 (VvSPL6)</i>  | VIT_01s0011g00130                        | Forward | GAAC TGAGGACTGC ACTGG   | 109               |
|   |  | Reverse | AGTCCTGCGATTGAGCTGA     |                   |
| <i>Small Nuclear RNA U6 (VvU6)</i>                        | VIT_06s0009g02760                        | Forward | ACAGAGAAGATTAGCATGGC    | 58                |
|   |  | Reverse | ACCATTCTCGATTGTGC       |                   |
| <i>Ubiquitin (VvUBI)</i>                                  | VIT_16s0098g01190<br>Perrone et al. 2012 | Forward | TCTGAGGCTTCGTGGTGGTA    | 99                |
|   |  | Reverse | AGGC GTGCATAACATTGCG    |                   |
| <i>Actin (VvACT1)</i>                                     | VIT_04s0044g00580<br>Perrone et al. 2012 | Forward | GCCCCTCGTCTGTGACAATG    | 101               |
|   |  | Reverse | CCTTGGCCGACCCACAATA     |                   |

**Table S3.** Conserved and known miRNAs identified by high-throughput SOLiD sequencing in *Vitis vinifera* cv. Cabernet Sauvignon (CS) and M4 root (R) and leaf (L) samples upon irrigated (IRR) and drought stress (DS) conditions. The relative number of reads was calculated by dividing the number of reads of each miRNA with the total number of reads obtained for each cDNA library (see the Methods section for further details).

| miRbase ID  | miRNA synonyms  | miRNA family | RELATIVE NUMBER OF READS |       |       |       |                        |       |       |       |
|-------------|---|--------------|--------------------------|-------|-------|-------|------------------------|-------|-------|-------|
|             |   |              | CS auto-grafted plants   |       |       |       | M4 auto-grafted plants |       |       |       |
|             |   |              | IRR L                    | IRR R | DS L  | DS R  | IRR L                  | IRR R | DS L  | DS R  |
| vvi-miR156b | vvi-miR156c vvi-miR156d                                     | miR156       | 496                      | 880   | 655   | 1894  | 528                    | 551   | 936   | 752   |
| vvi-miR156e |   |              | 0                        | 109   | 0     | 0     | 4                      | 9     | 0     | 2     |
| vvi-miR156f | vvi-miR156g vvi-miR156i                                     |              | 4363                     | 400   | 758   | 2027  | 1651                   | 492   | 602   | 2688  |
| vvi-miR159a |   | miR159       | 0                        | 101   | 0     | 59    | 1                      | 37    | 28    | 3     |
| vvi-miR159c |   |              | 20088                    | 41022 | 12976 | 49683 | 15970                  | 45946 | 18164 | 50821 |
| vvi-miR160a | vvi-miR160b   | miR160       | 161                      | 299   | 221   | 547   | 175                    | 145   | 245   | 127   |
| vvi-miR160c | vvi-miR160d vvi-miR160e                                     |              | 1644                     | 2615  | 1617  | 3595  | 1864                   | 1678  | 2424  | 676   |
| vvi-miR162  |   | miR162       | 31101                    | 12696 | 32202 | 21483 | 21361                  | 11915 | 10352 | 24036 |
| vvi-miR164a | vvi-miR164c vvi-miR164d                                     | miR164       | 5233                     | 3208  | 9534  | 5341  | 7767                   | 3088  | 1332  | 1739  |
| vvi-miR164b |   |              | 105                      | 44    | 57    | 0     | 300                    | 15    | 11    | 78    |
| vvi-miR166a | vvi-miR166b   | miR166       | 372                      | 1211  | 564   | 725   | 1043                   | 1143  | 1187  | 701   |
| vvi-miR166c | vvi-miR166d vvi-miR166e vvi-miR166f vvi-miR166g vvi-miR166h |              | 22532                    | 60207 | 73810 | 41427 | 76829                  | 58087 | 28974 | 54561 |
| vvi-miR167a |   | miR167       | 421                      | 246   | 100   | 547   | 203                    | 89    | 535   | 655   |
| vvi-miR167b | vvi-miR167d vvi-miR167e                                     |              | 20211                    | 6429  | 8962  | 9173  | 14204                  | 4086  | 6057  | 11469 |
| vvi-miR167c |   |              | 2199                     | 3567  | 1420  | 12561 | 1332                   | 5997  | 20421 | 1124  |
| vvi-miR168  |   | miR168       | 8391                     | 8043  | 4258  | 4572  | 6599                   | 5528  | 1231  | 2477  |
| vvi-miR169a | vvi-miR169c vvi-miR169j vvi-miR169k vvi-miR169s vvi-miR169w | miR169       | 644                      | 1283  | 690   | 459   | 928                    | 1644  | 1237  | 1190  |
| vvi-miR169b | vvi-miR169h   |              | 124                      | 24    | 149   | 30    | 165                    | 18    | 67    | 208   |
| vvi-miR169d |   |              | 1131                     | 2341  | 721   | 1835  | 2776                   | 5225  | 7550  | 2595  |
| vvi-miR169e |   |              | 449                      | 32    | 175   | 118   | 104                    | 82    | 28    | 214   |
| vvi-miR169f | vvi-miR169g   |              | 14631                    | 428   | 12854 | 3196  | 11894                  | 545   | 1399  | 18096 |

**Table S3.** Continued.

| miRBase ID  | miRNA synonyms  | miRNA family | RELATIVE NUMBER OF READS |       |      |      |                        |       |      |      |
|-------------|---|--------------|--------------------------|-------|------|------|------------------------|-------|------|------|
|             |   |              | CS auto-grafted plants   |       |      |      | M4 auto-grafted plants |       |      |      |
|             |   |              | IRR L                    | IRR R | DS L | DS R | IRR L                  | IRR R | DS L | DS R |
| vvi-miR169l |   | miR169       | 180                      | 113   | 94   | 44   | 70                     | 51    | 11   | 132  |
| vvi-miR169m | vvi-miR169n vvi-miR169p vvi-miR169q   |              | 237                      | 182   | 144  | 192  | 150                    | 91    | 22   | 289  |
| vvi-miR169o |   |              | 289                      | 36    | 141  | 74   | 108                    | 4     | 6    | 196  |
| vvi-miR169r | vvi-miR169u   |              | 129                      | 186   | 51   | 74   | 54                     | 221   | 139  | 255  |
| vvi-miR169t |   |              | 42                       | 44    | 10   | 30   | 63                     | 79    | 39   | 153  |
| vvi-miR169v |   |              | 93                       | 65    | 29   | 44   | 311                    | 218   | 306  | 310  |
| vvi-miR171a | vvi-miR171c vvi-miR171d vvi-miR171i   | miR171       | 1722                     | 3749  | 612  | 6939 | 625                    | 5003  | 7149 | 248  |
| vvi-miR171f |   |              | 4                        | 133   | 3    | 0    | 14                     | 111   | 84   | 4    |
| vvi-miR172c |   | miR172       | 75                       | 743   | 74   | 163  | 82                     | 837   | 1131 | 25   |
| vvi-miR172d |   |              | 1323                     | 2103  | 2225 | 1391 | 1642                   | 3617  | 2401 | 1348 |
| vvi-miR390  |   | miR390       | 137                      | 117   | 134  | 59   | 211                    | 196   | 178  | 53   |
| vvi-miR393a | vvi-miR393b   | miR393       | 121                      | 0     | 114  | 98   | 107                    | 1     | 90   | 10   |
| vvi-miR394a | vvi-miR394c   | miR394       | 139                      | 202   | 154  | 385  | 78                     | 113   | 184  | 73   |
| vvi-miR394b |   |              | 138                      | 198   | 153  | 370  | 76                     | 113   | 184  | 73   |
| vvi-miR395a | vvi-miR395b vvi-miR395c vvi-miR395d vvi-miR395e vvi-miR395f vvi-miR395g vvi-miR395h vvi-miR395i vvi-miR395j vvi-miR395k vvi-miR395l vvi-miR395m | miR395       | 1791                     | 73    | 733  | 621  | 583                    | 47    | 150  | 262  |
| vvi-miR396a |   | miR396       | 8601                     | 7966  | 2245 | 8197 | 3184                   | 5239  | 5193 | 4183 |
| vvi-miR396b |   |              | 3761                     | 4580  | 893  | 3817 | 1082                   | 3322  | 2435 | 1527 |

**Table S3.** Continued.

| miRBase ID  | miRNA synonyms  | miRNA family | RELATIVE NUMBER OF READS |        |       |        |                        |        |        |        |
|-------------|---|--------------|--------------------------|--------|-------|--------|------------------------|--------|--------|--------|
|             |   |              | CS auto-grafted plants   |        |       |        | M4 auto-grafted plants |        |        |        |
|             |   |              | IRR L                    | IRR R  | DS L  | DS R   | IRR L                  | IRR R  | DS L   | DS R   |
| vvi-miR396c | vvi-miR396d   | miR396       | 144836                   | 232688 | 72230 | 105492 | 80946                  | 165154 | 109141 | 102958 |
| vvi-miR397a |   | miR397       | 7589                     | 11316  | 13457 | 7546   | 3047                   | 34615  | 21953  | 2443   |
| vvi-miR398a |   | miR398       | 5051                     | 3313   | 3261  | 2382   | 3338                   | 5033   | 3460   | 814    |
| vvi-miR398b | vvi-miR398c   |              | 3190                     | 1768   | 5070  | 2811   | 1336                   | 2443   | 3705   | 1045   |
| vvi-miR399a | vvi-miR399h   | miR399       | 5744                     | 589    | 22259 | 1997   | 1445                   | 189    | 1777   | 4370   |
| vvi-miR399b | vvi-miR399c   |              | 8547                     | 1340   | 4642  | 1879   | 899                    | 821    | 1471   | 2327   |
| vvi-miR399e |   |              | 140                      | 24     | 1387  | 133    | 137                    | 26     | 106    | 403    |
| vvi-miR399g |   |              | 835                      | 137    | 3475  | 562    | 404                    | 40     | 273    | 628    |
| vvi-miR399i |   |              | 22730                    | 2740   | 24647 | 8138   | 4215                   | 825    | 2925   | 7393   |
| vvi-miR403a | vvi-miR403b vvi-miR403c vvi-miR403d vvi-miR403e vvi-miR403f | miR403       | 919                      | 916    | 647   | 2382   | 1201                   | 806    | 1705   | 1316   |
| vvi-miR408  |   | miR408       | 4368                     | 1760   | 5076  | 2841   | 3556                   | 1492   | 1878   | 2229   |
| vvi-miR447b |   | miR447       | 261                      | 73     | 29    | 30     | 0                      | 0      | 0      | 1      |
| vvi-miR477  |   | miR477       | 141                      | 254    | 47    | 89     | 39                     | 191    | 357    | 143    |
| vvi-miR479  |   | miR479       | 18                       | 307    | 24    | 252    | 121                    | 60     | 123    | 58     |
| vvi-miR482  |   | miR482       | 23727                    | 54517  | 15415 | 83121  | 13602                  | 72937  | 111270 | 7367   |
| vvi-miR535a | vvi-miR535b vvi-miR535c                                     | miR535       | 49634                    | 15662  | 43924 | 31367  | 62976                  | 9561   | 13216  | 53725  |
| vvi-miR828a |   | miR828       | 256                      | 65     | 260   | 118    | 130                    | 37     | 50     | 249    |
| vvi-miR2111 |   | miR2111      | 298                      | 484    | 149   | 829    | 236                    | 366    | 318    | 259    |
| vvi-miR2950 |   | miR2950      | 1053                     | 1445   | 418   | 621    | 378                    | 992    | 412    | 1233   |
| vvi-miR3623 |   | miR3623      | 11311                    | 7058   | 6072  | 8626   | 7456                   | 7727   | 11105  | 9254   |
| vvi-miR3624 |   | miR3624      | 15787                    | 20202  | 15396 | 22208  | 78452                  | 50916  | 66388  | 64638  |

**Table S3.** Continued.

| miRBase<br>ID | miRNA synonyms                         | miRNA<br>family | RELATIVE NUMBER OF READS |          |         |         |                        |          |         |         |
|---------------|--|-----------------|--------------------------|----------|---------|---------|------------------------|----------|---------|---------|
|               |  |                 | CS auto-grafted plants   |          |         |         | M4 auto-grafted plants |          |         |         |
|               |  |                 | IRR<br>L                 | IRR<br>R | DS<br>L | DS<br>R | IRR<br>L               | IRR<br>R | DS<br>L | DS<br>R |
| vvi-miR3626   |  | miR3626         | 333                      | 359      | 66      | 163     | 0                      | 0        | 33      | 0       |
| vvi-miR3629a  | vvi-miR3629b vvi-miR3629c              | miR3629         | 17                       | 0        | 21      | 15      | 208                    | 1        | 33      | 43      |
| vvi-miR3631a  | vvi-miR3631b vvi-miR3631c vvi-miR3631d | miR3631         | 29                       | 52       | 15      | 148     | 24                     | 45       | 28      | 12      |
| vvi-miR3632   |  | miR3632         | 1333                     | 3220     | 1248    | 4350    | 1480                   | 4159     | 5700    | 1635    |
| vvi-miR3633a  |  | miR3633         | 31                       | 157      | 49      | 266     | 3                      | 233      | 50      | 7       |
| vvi-miR3634   |  | miR3634         | 14537                    | 13019    | 5860    | 7442    | 12245                  | 14546    | 12877   | 13288   |
| vvi-miR3636   |  | miR3636         | 369                      | 428      | 257     | 577     | 1813                   | 2021     | 2078    | 987     |
| vvi-miR3637   |  | miR3637         | 72                       | 149      | 93      | 296     | 271                    | 239      | 518     | 99      |
| vvi-miR3638   |  | miR3638         | 80                       | 77       | 303     | 281     | 117                    | 48       | 0       | 60      |
| vvi-miR3639   |  | miR3639         | 1                        | 0        | 0       | 0       | 151                    | 100      | 279     | 121     |
| vvi-miR3640   |  | miR3640         | 54                       | 186      | 50      | 266     | 233                    | 409      | 813     | 176     |

**Table S4.** Putative novel miRNAs identified by high-throughput SOLiD sequencing in CS and M4 root (R) and leaf (L) samples upon irrigated (IRR) and drought stress (DS) conditions. The relative number of reads was calculated by dividing the number of reads of each miRNA with the total number of reads obtained for each cDNA library (see the Methods section for further details).

| novel<br>miRNA<br>ID | Chr | Start        | End          | Strand | 5'-3'<br>Sequence               | Length<br>(nt) | Hairpin<br>length<br>(nt) | Hairpin<br>minimum<br>free<br>energy<br>(ΔG kcal<br>mol <sup>-1</sup> ) | RELATIVE NUMBER OF READS |          |         |         |                        |          |         |         |
|----------------------|-----|--------------|--------------|--------|---------------------------------|----------------|---------------------------|---|--------------------------|----------|---------|---------|------------------------|----------|---------|---------|
|                      |     |              |              |        |                                 |                |                           |   | CS auto-grafted plants   |          |         |         | M4 auto-grafted plants |          |         |         |
|                      |     |              |              |        |                                 |                |                           |   | IRR<br>L                 | IRR<br>R | DS<br>L | DS<br>R | IRR<br>L               | IRR<br>R | DS<br>L | DS<br>R |
| n_22                 | 5   | 6841977      | 6841996      | -      | TAGACCAT<br>GCATTTTC<br>ATT     | 20             | 111                       | -28.90  | 0                        | 104      | 783     | 47      | 871                    | 905      | 258     | 1432    |
| n_47                 | 4   | 1975549<br>3 | 1975551<br>4 | -      | TTTCCCCA<br>ATGCCGCC<br>CATTCC  | 22             | 173                       | -71.80  | 25795                    | 15911    | 8323    | 8114    | 5177                   | 9260     | 5318    | 12422   |
| n_55                 | 4   | 2281492<br>2 | 2281493<br>9 | +      | ATTCTTATC<br>GATIGATA<br>G      | 18             | 146                       | -37.30  | 0                        | 0        | 0       | 126     | 0                      | 0        | 0       | 0       |
| n_96                 | 2   | 1792380<br>4 | 1792382<br>2 | +      | TTAGATTA<br>CATTAGTA<br>AGT     | 19             | 101                       | -23.30  | 0                        | 0        | 155     | 0       | 84                     | 6        | 0       | 0       |
| n_107                | Un  | 11734039     | 11734059     | +      | GTGCTCTC<br>TCTCGCTG<br>TCATA   | 21             | 146                       | -55.90  | 227                      | 0        | 136     | 0       | 0                      | 0        | 0       | 0       |
| n_135                | 7   | 1507765<br>1 | 1507766<br>9 | -      | CCGGGTAG<br>ATATGTTG<br>GGA     | 19             | 101                       | -38.00  | 0                        | 62       | 0       | 126     | 0                      | 29       | 150     | 0       |
| n_191                | Un  | 4244887<br>2 | 4244889<br>4 | +      | GACTTAGG<br>AAGAACGC<br>ACCTGCA | 23             | 163                       | -86.20  | 109                      | 0        | 0       | 0       | 0                      | 0        | 0       | 0       |
| n_221                | 5   | 2474219<br>4 | 2474221<br>5 | -      | TTTGTG<br>CTGGTCAT<br>CTAGTC    | 22             | 149                       | -52.60  | 580                      | 17       | 0       | 94      | 103                    | 0        | 0       | 0       |
| n_231                | 3   | 1700358<br>5 | 1700360<br>5 | +      | TGCCAAGA<br>AGCACATT<br>CCTCC   | 21             | 81                        | -67.00  | 0                        | 0        | 0       | 5630    | 0                      | 26609    | 0       | 0       |
| n_284                | 14  | 2733086<br>8 | 2733088<br>7 | -      | ATATATCTG<br>ACATACCA<br>ATT    | 20             | 108                       | -27.80  | 6                        | 109      | 7       | 94      | 11                     | 75       | 107     | 21      |
| n_290                | 2   | 855711       | 855731       | -      | AGAGCTTT<br>CTTCAGTC<br>CACTC   | 21             | 212                       | -80.10  | 6                        | 0        | 0       | 0       | 14                     | 0        | 112     | 0       |
| n_306                | 8   | 2230823<br>9 | 2230825<br>9 | +      | ATGTATTTG<br>AGGGAAA<br>GCAA    | 21             | 153                       | -61.40  | 103                      | 0        | 5       | 0       | 0                      | 13       | 0       | 27      |

**Table S4.** Continued.

| novel<br>miRNA<br>ID | Chr | Start        | End          | Strand | 5'-3'<br>Sequence                | Length<br>(nt) | Hairpin<br>length<br>(nt) | Hairpin<br>minimum<br>free<br>energy<br>(ΔG kcal<br>mol <sup>-1</sup> ) | RELATIVE NUMBER OF READS |          |         |         |                        |          |         |         |
|----------------------|-----|--------------|--------------|--------|----------------------------------|----------------|---------------------------|---|--------------------------|----------|---------|---------|------------------------|----------|---------|---------|
|                      |     |              |              |        |                                  |                |                           |   | CS auto-grafted plants   |          |         |         | M4 auto-grafted plants |          |         |         |
|                      |     |              |              |        |                                  |                |                           |   | IRR<br>L                 | IRR<br>R | DS<br>L | DS<br>R | IRR<br>L               | IRR<br>R | DS<br>L | DS<br>R |
| n_312                | 6   | 1557559<br>1 | 1557561<br>2 | +      | GGAGTGAC<br>CTGAGAAC<br>CACATG   | 22             | 157                       | -72.80  | 0                        | 0        | 0       | 0       | 94                     | 0        | 0       | 0       |
| n_315                | 11  | 1504204      | 1504224      | -      | GCTTACTC<br>CCTATCTG<br>TCACC    | 21             | 110                       | -47.20  | 0                        | 584      | 0       | 55      | 0                      | 315      | 50      | 0       |
| n_327                | Un  | 11820804     | 11820824     | +      | TTGACAAA<br>GAGAGAG<br>AGCACAA   | 21             | 117                       | -52.90  | 0                        | 0        | 0       | 0       | 202                    | 9        | 0       | 0       |
| n_337                | 1   | 2155013<br>3 | 2155015<br>6 | -      | TGTTTGGT<br>TAGTGATG<br>GGATAAGA | 24             | 90                        | -36.30  | 0                        | 0        | 0       | 131     | 0                      | 0        | 0       | 0       |
| n_346                | 6   | 1995075<br>5 | 1995077<br>5 | -      | GCTGGAAG<br>CCGATGGG<br>GGACC    | 21             | 145                       | -59.10  | 0                        | 0        | 241     | 0       | 0                      | 0        | 0       | 0       |
| n_401                | 14  | 2646374<br>4 | 2646376<br>5 | +      | CCTCTCTC<br>TTCTCCTG<br>TCAACA   | 22             | 95                        | -43.90  | 180                      | 26       | 109     | 0       | 0                      | 27       | 11      | 0       |
| n_416                | 12  | 1094032<br>4 | 1094034<br>2 | +      | CCTTGCAA<br>AATTTCG<br>CCA       | 19             | 151                       | -67.70  | 0                        | 0        | 0       | 68      | 0                      | 0        | 142     | 4       |
| n_471                | 9   | 2182245<br>1 | 2182247<br>4 | +      | AGTATATTT<br>GCATCAAA<br>TTTGTCA | 24             | 166                       | -60.00  | 90                       | 60       | 50      | 113     | 21                     | 32       | 0       | 36      |
| n_479                | 1   | 3865640      | 3865661      | +      | TCTTACCA<br>ACACCTCC<br>CATTCC   | 22             | 156                       | -55.80  | 107728                   | 43575    | 54805   | 33779   | 49632                  | 51423    | 28463   | 46827   |
| n_520                | 17  | 3046335      | 3046355      | -      | GCTCTCTA<br>GACTTCTG<br>TCATC    | 21             | 101                       | -48.10  | 4257                     | 269      | 1188    | 581     | 3156                   | 320      | 671     | 3030    |
| n_529                | 1   | 2155013<br>4 | 2155015<br>7 | -      | ATGTTTGG<br>TTAGTGAT<br>GGGATAAG | 24             | 90                        | -36.30  | 47                       | 177      | 9       | 0       | 0                      | 0        | 0       | 3       |
| n_532                | 6   | 8159628      | 8159648      | +      | CGCTATCT<br>ATCCTGAG<br>TTTCA    | 21             | 153                       | -65.00  | 540                      | 294      | 151     | 92      | 88                     | 232      | 96      | 101     |
| n_579                | 8   | 1994927<br>6 | 1994929<br>7 | -      | CACATATA<br>ATTTTTCC<br>CGTCA    | 22             | 156                       | -47.90  | 124                      | 141      | 56      | 0       | 87                     | 42       | 65      | 93      |

**Table S4.** Continued.

| novel<br>miRNA<br>ID | Chr | Start   | End     | Strand | 5'-3'<br>Sequence              | Length<br>(nt) | Hairpin<br>length<br>(nt) | Hairpin<br>minimum<br>free<br>energy<br>(ΔG kcal<br>mol <sup>-1</sup> ) | RELATIVE NUMBER OF READS |          |         |         |                        |          |         |         |
|----------------------|-----|---------|---------|--------|--------------------------------|----------------|---------------------------|---|--------------------------|----------|---------|---------|------------------------|----------|---------|---------|
|                      |     |         |         |        |                                |                |                           |   | CS auto-grafted plants   |          |         |         | M4 auto-grafted plants |          |         |         |
|                      |     |         |         |        |                                |                |                           |   | IRR<br>L                 | IRR<br>R | DS<br>L | DS<br>R | IRR<br>L               | IRR<br>R | DS<br>L | DS<br>R |
| n_581                | 5   | 6820787 | 6820808 | -      | TTACACAG<br>AGAGATGA<br>CGGTGG | 22             | 99                        | -49.20  | 0                        | 222      | 35      | 0       | 41                     | 376      | 90      | 65      |
| n_622                | 4   | 5357009 | 5357030 | -      | GCTCATT<br>CTCTTTCT<br>GTCAGC  | 22             | 116                       | -58.90  | 2241                     | 11817    | 1342    | 5846    | 4374                   | 5757     | 6809    | 3918    |
| n_710                | 17  | 5927890 | 5927911 | +      | TCCCAGGA<br>GAGATGGC<br>ACCTGC | 22             | 149                       | -62.90  | 3156                     | 69       | 1311    | 378     | 1027                   | 81       | 20      | 1349    |

**Table S5.** Target transcripts of the novel candidate miRNAs analysed in this study. For each novel miRNA, the related target genes with the corresponding description and functional annotation are reported.

| Novel miRNA ID | Target Gene ID (VvGDB 12X V1 CRIBI) | Gene Description   | GO Functional Category                   |
|----------------|-------------------------------------|--|--|
| n_22           | VIT_12s0035g00260                   | Calmodulin binding protein (MLO16)                       | Stress response                          |
| n_47           | VIT_09s0096g00830                   | NBS-LRR defense protein                                  | Stress response                          |
| n_231          | VIT_00s0480g00100                   | Polyphenol oxidase, chloroplastic-like                   | Secondary metabolism                     |
| n_479          | VIT_09s0002g04950                   | NBS-LRR defense protein RPS5-like                        | Stress response                          |
|                | VIT_11s0037g01270                   | NBS-LRR defense protein                                  | Stress response                          |
| n_520          | VIT_06s0004g06440                   | zinc finger domain transcription factor                  | Regulation of transcription              |
| n_622          | VIT_05s0020g03140                   | Hexose transporter (HT5)                                 | Sugar transport                          |
|                | VIT_03s0038g00720                   | NADH dehydrogenase                                       | Energy metabolism                        |
| n_191          | VIT_13s0067g02480                   | CBL interacting protein kinase 13 (CIPK13)               | ABA-mediated signaling pathway           |
| n_327          | VIT_01s0011g00130                   | Squamosa Promoter binding protein-Like 6 (SPL6)          | Regulation of transcription (SBP family) |
|                | VIT_01s0010g03710                   | Squamosa Promoter binding protein-Like 12 (SPL12)        | Regulation of transcription (SBP family) |
| n_346          | VIT_06s0009g03800                   | Uncharacterized protein: PHD finger transcription factor | Regulation of transcription (DDT family) |
|                | VIT_08s0007g06560                   | putative serine/threonine kinase                         | Signaling pathway                        |
| n_55           | VIT_12s0059g00450                   | Uncharacterized protein: DNA topoisomerase I             | Nucleic acid metabolism                  |
|                | VIT_04s0023g01900                   | Uncharacterized protein: AAA-type ATPase                 | Transport                                |

**Table S6.** Target transcripts of the conserved/known miRNAs analysed in this study. For each conserved or known miRNA, the related target genes are reported with the corresponding description, functional annotation and reference.

| miRNA ID<br>(miRBase<br>v.22) | Target Gene ID<br>(VvGDB 12X V1<br>CRIBI) | Gene Description                                    | GO Functional Category                         | Reference <sup>a</sup>                                       |
|-------------------------------|---|---|--|--|
| miR156b,c,d                   | VIT_04s0008g07340                         | Zinc finger protein CONSTANS 5-like (CONSTANS like) | Development                                    | Pantaleo <i>et al.</i> , 2010                                |
| miR393a                       | VIT_14s0068g01330                         | Auxin responsive factor TIR1-like                   | Auxin-mediated signalling pathway              | Belli Kullan <i>et al.</i> , 2015                            |
| miR396c,d                     | VIT_00s0494g00010                         | Growth regulating factor 1 like (GRL1 like)         | Regulation of transcription (GRF family)       | Belli Kullan <i>et al.</i> , 2015;                           |
| miR3624                       | VIT_00s0194g00340                         | Metallothionein                                     | Cellular homeostasis and Stress response       | Carra <i>et al.</i> , 2009;<br>Pantaleo <i>et al.</i> , 2010 |
| miR159c                       | VIT_00s0287g00040                         | GATA transcription factor 26-like (GATA)            | Regulation of transcription (C2C2-GATA family) | Pantaleo <i>et al.</i> , 2010                                |
|                               | VIT_09s0002g08370                         | Topless-related protein 1 (TPR1)                    | Auxin-mediated signaling pathway               |  |
|                               | VIT_19s0090g00590                         | Myb-like transcription factor (MYB)                 | Regulation of transcription (MYB family)       |  |

<sup>a</sup> scientific works where the target genes were *in silico* predicted and/or validated by either degradome or 5'RACE technique.