

Supporting Online Material

Figures S1-5

Figure S1. The stem-loop structures of *MIR172* loci from *A. thaliana* (At) and *A. arenosa* (Aa) (*MIR172a*, *b*, *d*, and *e*).

Figure S2. Sequence alignment of *A. arenosa MIR172a* locus with the corresponding *A. thaliana MIR172a*.

Figure S3. Sequence alignment of *A. arenosa MIR172b* locus with the corresponding *A. thaliana MIR172b*.

Figure S4. Sequence alignment of *A. arenosa MIR172d* locus with the corresponding *A. thaliana MIR172d*.

Figure S5. Sequence alignment of *A. arenosa MIR172e* locus with the corresponding *A. thaliana MIR172e*.

Tables 1-5

Table S1. DNA PCR primers used to amplify *A. arenosa* miRNA 172 loci

| | Forward primers | Reverse primers |
|---------|--------------------------------|--------------------------------|
| MIR172a | 5'-TGTGAAAATAGCTGCCAACTTGC-3' | 5'-GATCTCACCAGGTCTTTCTCTGGT-3' |
| MIR172b | 5'-TCGGGACCAAGTAATAACCCAGA-3' | 5'-TCTTCACTTGACCTCTCACTCC-3' |
| MIR172d | 5'-TGTCGTCTAACGGATAGCCAAGAG-3' | 5'-ACACGAAAAACAAGGGGTTTGGT-3' |
| MIR172e | 5'-AGCCTTTGGCTTCTGTTCTGAC-3' | 5'-TTCTCTGCCATCGTTGTTTACTTG-3' |

Table S2. DNA primers used for RACE-PCR amplification

| Primer Name | Sequence |
|--------------------|------------------------------|
| nmiR172bRACE-3 | CAAGTTGTCGGCCGGATCCATGGAAGA |
| nmiR172bRACE-nest3 | TGCTGCATCAACAATCGACGGCTACA |
| nmiR172bRACE-5 | GTGCTGCACCTACAAACAACGACAGATG |
| nmiR172bRACE-nest5 | CCATGGATCCGCCGACAACCTTGTAT |

Table S3. DNA primers used for SSCP and RT-PCR analyses

| Locus | Forward primers | Reverse primers |
|---------------|-----------------------------------|--------------------------|
| At5g04240sscp | CCATTGCGAAAAGGCTGAAGAA | CCACATCCATCGACCTTGCATA |
| At5g04250sscp | GGAAAATCAAGGGGAAGCCATT | TCTGCTCATCCTCTGACGGTAA |
| At5g04260sscp | TGATTCACCGAGCTTCTGAGCA | GATCTCACCTTTGGTTCCCTCCA |
| At5g04270sscp | TCGATGCAGAAAAGCTAATACCG | TGGGACTCTCCTTTGTTGGCATA |
| At5g04280sscp | TGCATGGAAGGGACTTTGGTGA | GACCACCACCAGCAGAAGGACA |
| At5g04290sscp | GCAGAGGAGGAAGGTCGTTTGA | GCCCCATGCATTATCAGCTTCT |
| At5g04310sscp | CATGGCCGGTGATTTGGATATT | GGCGTCGTCACCAATACCTTCT |
| At5g04320sscp | CATCCGAAAGCAGGCATGAAAC | GCATTTTCGTCGCATCTTTACTC |
| At5g04330sscp | TTCTCAACTCGCCCGTTTATCC | TTTGGCGTCGGATGAGAAAAC |
| At5g04340sscp | CTACTCACACCGCCTCCGTTCT | CGTGACCTTGACCTGCCTTCTT |
| At5g04347sscp | TCTTGGTCAAAAATGAGCTGAACA | AGGTGTCTCGGGATGTTGTGTG |
| At5g04350sscp | CGATTGTTATTGGCTTATGCATTG | TCATCCCATTGGTACTTTTTGGTG |
| At5g04360sscp | GGCACTGACACTGACACCAACA | CTGGAGACTTCGAGCGACATAGG |
| At1g66690sscp | ACCCCGGCGCTAAAGTATCTTCT | TCATTGTAGCGGATTTCCGGTTGT |
| At1g66700sscp | CTGCATTCCGGGTTTGGAGTTAT | GGGAAGTTCACAATCGAGGCTTT |
| At5g39610sscp | GACCGTCAAGCAACAGCTTCATT | GCCGATGGTACAAAGGTTCCAAT |
| At5g67180sscp | CCGGTTCGTCGGTAAAACGTAAC | CGTGTCAAACGGGATGAGTCAAT |
| At2g28550Aa | CGG AGC ATG CCC GAG TTA TAA TAA T | TTGCATTGTCATTGGGAGGTTG |
| At4g36920Aa | GGA TTA GGT CAA ACC GGA ATG C | CTGCAGCCAATTTTGATGAGGA |
| At5g60120Aa | CTT CCT TCT GCG TTC TTT TCA CC | GCGGTTTCATGAGATTGTGTTGG |

Table S4. DNA primers used for genomic bisulfite sequencing

| Name of the primers | Sequences |
|---------------------|---------------------------------|
| AamiR172bBSP-F | GTGTTTATTTGAGAATAAGTTAGATTTAGT |
| AamiR172bBSP-R | AAAATAAAATATTTATAAAAACATACAATA |
| AamiR172bBSP-Fnest | TTATTTGAGAATAAGTTAGATTTAGTGTT |
| AamiR172bBSP-Rnest | CCAAATATTCATTAATATAATAACTATC |
| AtmiR172bBSP-F | TATTTATTTGAGAATAAGGGATATTTAGTG |
| AtmiR172bBSP-R | AAAAAATAAAATATAATATTTATAAAAACA |
| AtmiR172bBSP-Fnest | ATTATTAATAAAAAGAAATATTGTGATATTT |
| AtmiR172bBSP-Rnest | CCATATTTATAATTCATTATCTCTAAATC |

Table S5. Percentage of methylated CpG sites in the promoter regions of *A. thaliana* (At) and *A. arenosa* (Aa) *MIR172b* loci

| | <i>AtMIR172b</i> | | | | <i>AaMIR172b</i> | | | | Methylation (%) |
|---------|------------------|--------|--------|--------|------------------|--------|--|--|-----------------|
| | CG (1) | CG (2) | CG (3) | CG (4) | CG (1) | CG (2) | | | |
| At | 0/50 | 0/50 | 0/50 | 0/50 | | | | | 0 |
| Aa | | | | | 9/50 | 5/50 | | | 18 |
| Allo-At | 0/50 | 0/50 | 0/50 | 0/50 | | | | | 0 |
| Allo-Aa | | | | | 19/50 | 15/50 | | | 38 |
| As-At | 0/50 | 0/50 | 0/50 | 0/50 | | | | | 0 |
| As-Aa | | | | | 25/50 | 20/50 | | | 50 |

Note: % of methylation = total number of sequences containing methylated CG sites/total number of sequences. There are four CG sites in *AtMIR172b* and two in *AaMIR172b*.

Allo-At, AlloAa, As-At, and As-Aa indicate *A. thaliana* (At) and *A. arenosa* (Aa) alleles in resynthesized allotetraploid (Allo) and natural allotetraploid *A. suecica* (As), respectively.

The CHG site was not methylated (data not shown)

ND: not determined.