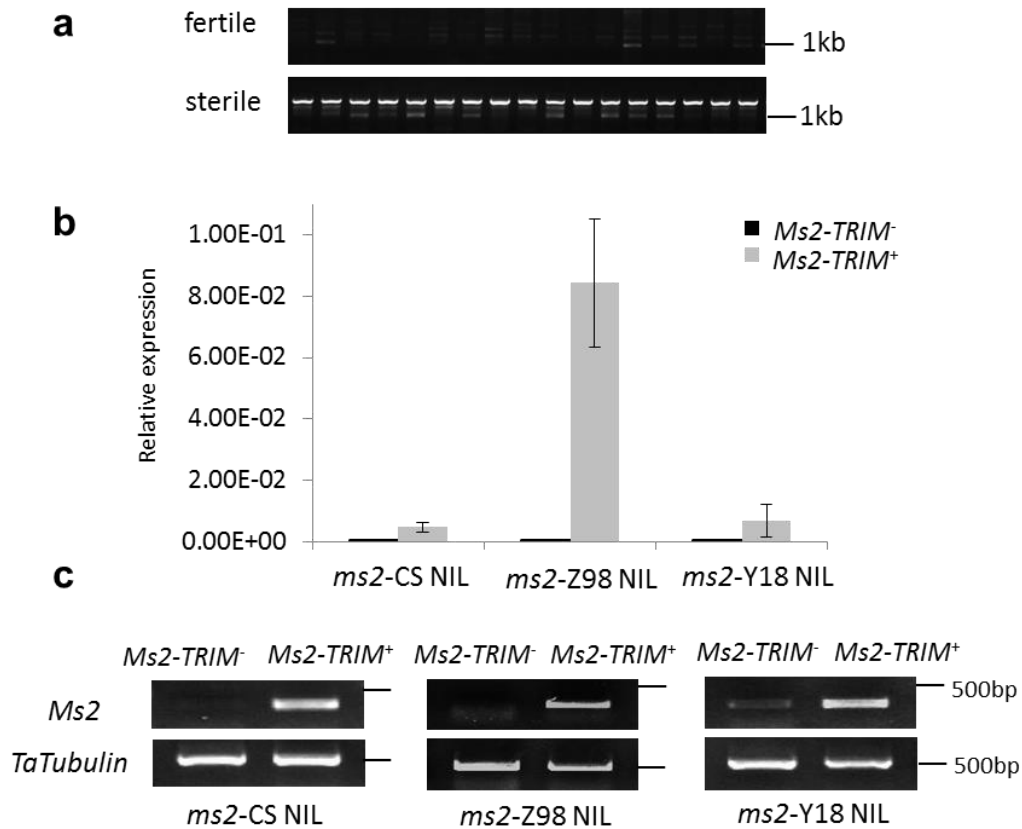


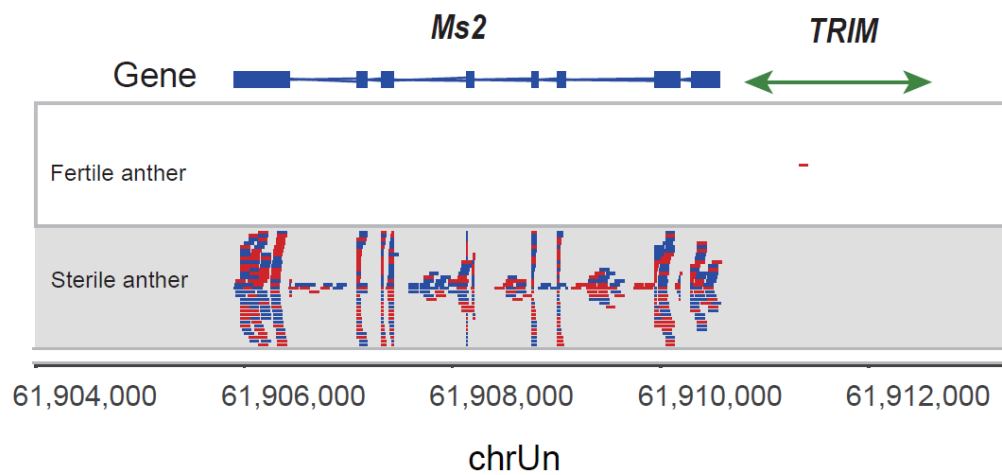
Supplementary Figures

Supplementary Fig. 1



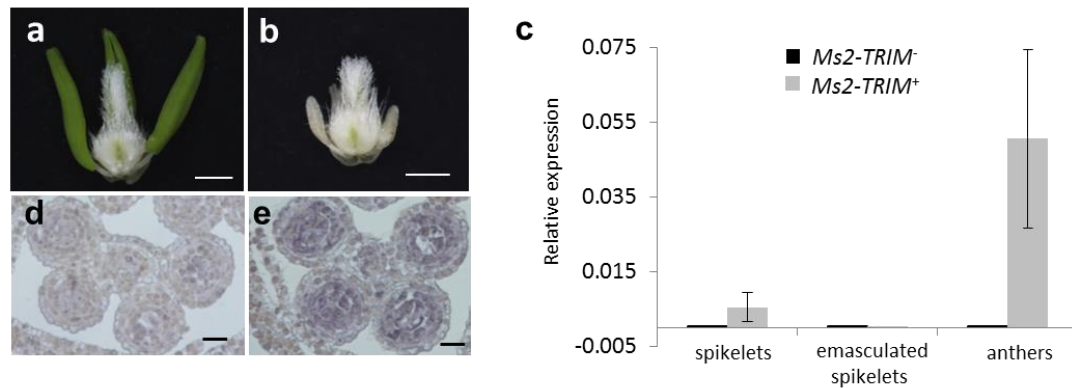
Supplementary Figure 1 | *Ms2* polymorphism and expression pattern analysis. (a) TRIM insertion status in 17 different *ms2* NILs detected by a PCR marker amplifying a portion of the TRIM element sequence and *Ms2* promoter. (b, c) *Ms2* expression in the anthers of WT (*Ms2-TRIM⁻*, fertile) and *ms2* mutant (*Ms2-TRIM⁺*, sterile) NIL plants in three genetic backgrounds (Chinese Spring, Zhengmai98, and Yumai18).

Supplementary Fig. 2



Supplementary Figure 2 | The reads distribution across the TRIM element and gene no.14. RNA-seq reads from the anther libraries from the *ms2* mutant and wild type wheat were mapped to an artificial wheat genome (Chinese Spring) with the *Ms2*-*TRIM* sequence inserted in front of gene no.14. The plot is adopted from SeqMonk, and the red and blue blocks represent reads mapped to forward mapping and reverse strands, separately.

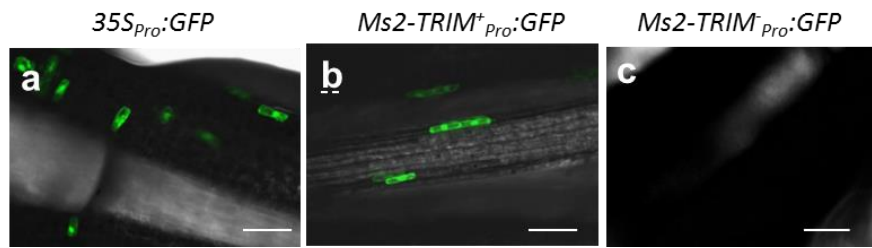
Supplementary Fig. 3



Supplementary Figure 3 | The phenotype of Ai-Bai wheat and *Ms2* expression analysis. Phenotypes of floral organs, following the removal of the palea and lemma, of tall-fertile progeny of Ai-Bai wheat ($-Ms2-TRIM$) (a) and dwarf-sterile progeny of Ai-Bai wheat ($Rht-D1c/Ms2-TRIM^+$) (b). (c) *Ms2* expression in three reproductive organs (spikelets, emasculated spikelets, anthers) of WT ($Ms2-TRIM^-$, fertile) and *ms2* mutant ($Ms2-TRIM^+$, sterile) plants in the Yanzhan1 genetic background. (d, e) *In situ* hybridization analysis (antisense probe) of *Ms2* in cross-sections of late prophase stage anthers from WT (d) and *ms2* mutant (e) plants.

Bars = 500 μm in (a) and (b), and 50 μm in (d) and (e).

Supplementary Fig. 4

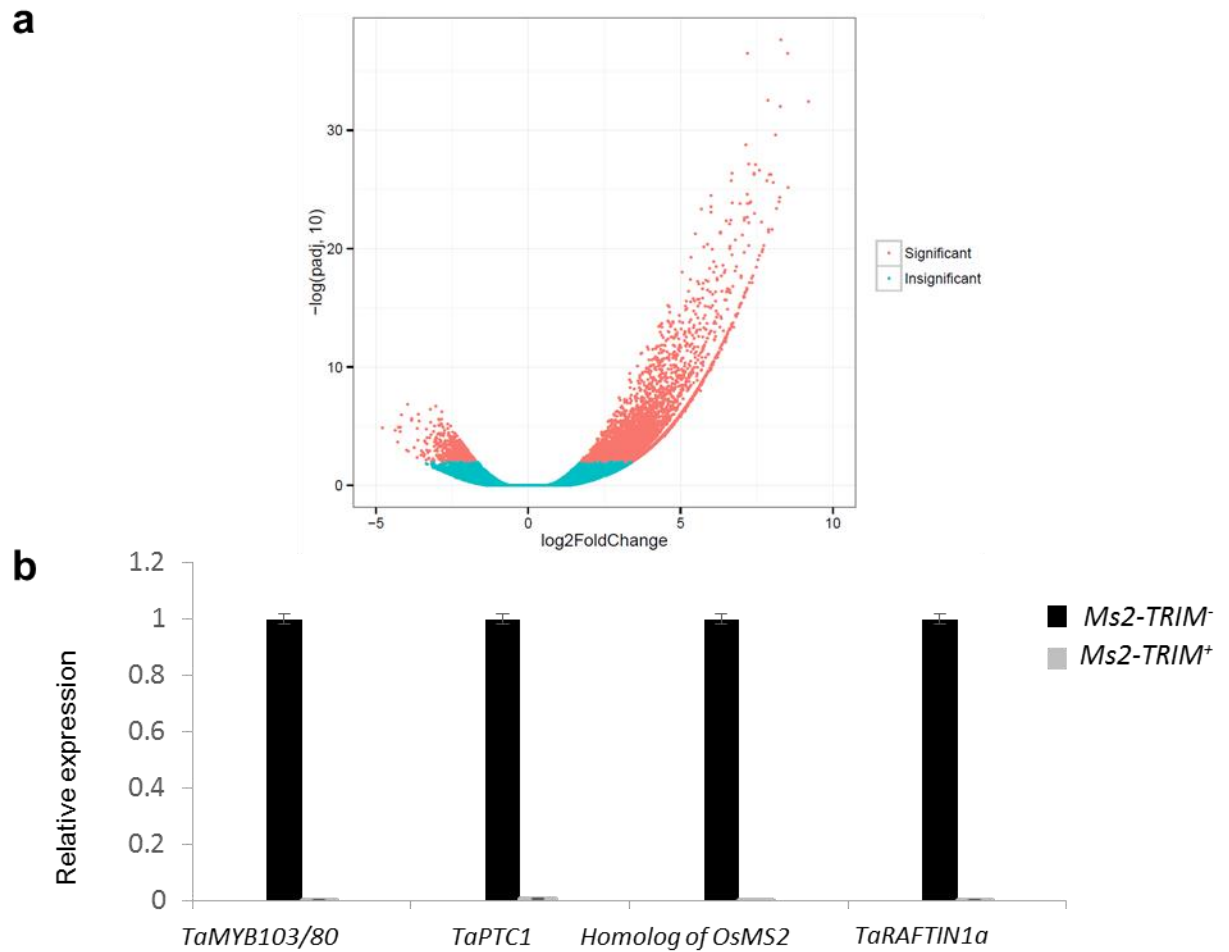


Supplementary Figure 4 | Activity analysis of the *Ms2* promoter.

(a-c) GFP fluorescence in anther transiently expressing **35S_{Pro}:GFP** (a), **Ms2-TRIM⁺_{Pro}:GFP** (b), and **Ms2-TRIM⁻_{Pro}:GFP** (c).

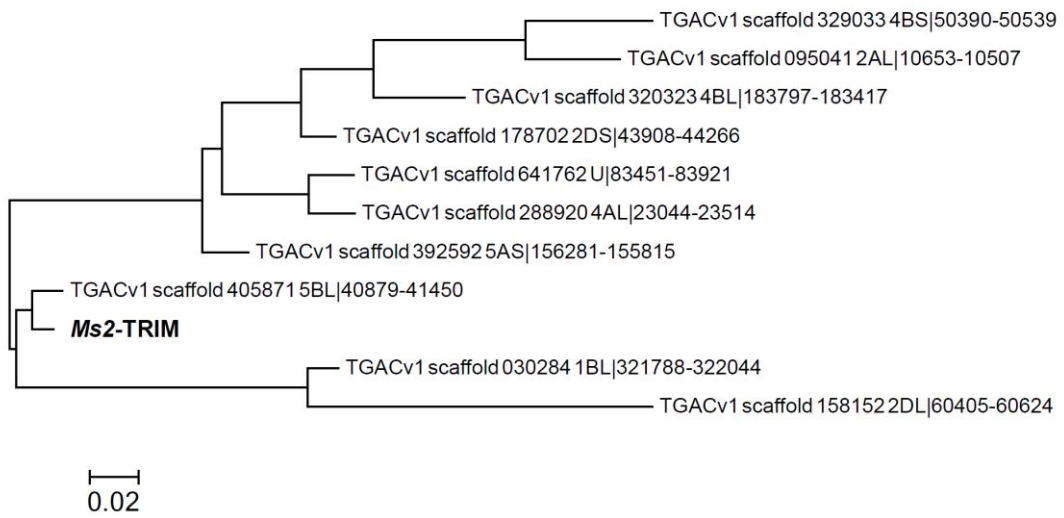
Bars = 100 μ m in (a) to (c).

Supplementary Fig. 5



Supplementary Figure 5 | Differentially expressed genes between *ms2* mutant vs. WT. (a) Volcano plot of differentially expressed genes between *ms2* mutant vs. WT anthers. **(b)** The relative expression of *TaMYB80/MYB103*, *TaPTC1*, *Homolog of OsMS2* and *TaRAFTIN1* in WT and *ms2* mutant plants.

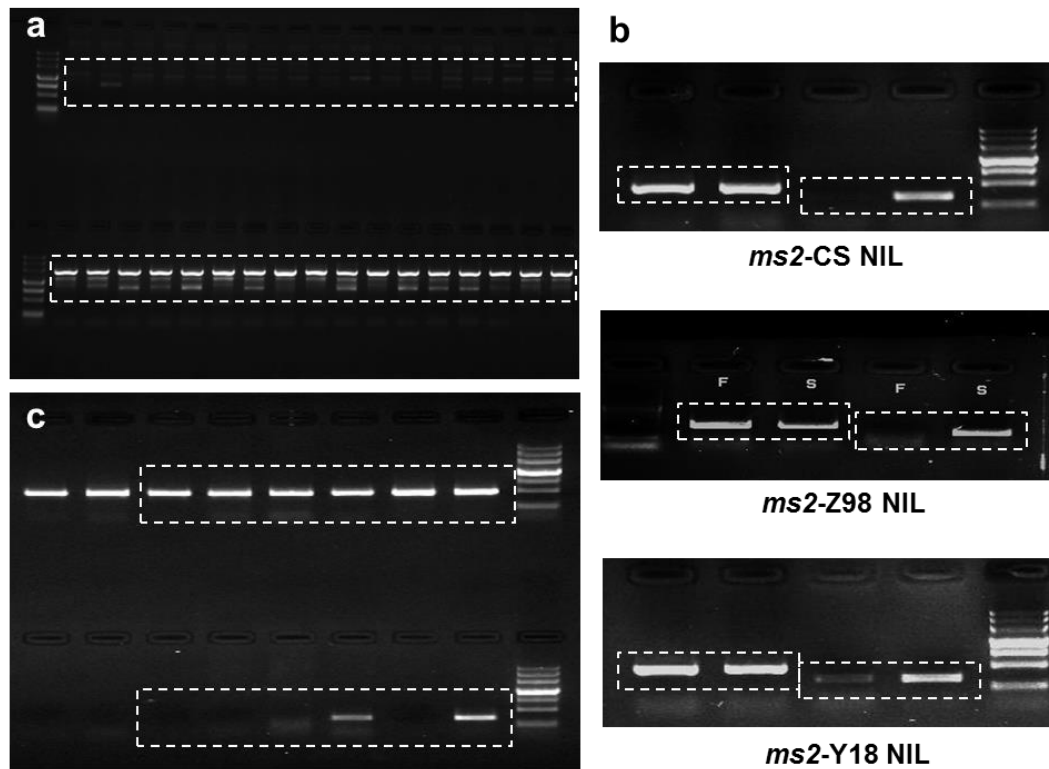
Supplementary Fig. 6



Supplementary Figure 6 | Phylogenetic tree of *MS2-TRIM* and its hits in the hexaploid wheat genome (Chinese Spring).

Unrooted Neighbor-Joining phylogeny of the *Ms2* promoter TRIM based on the LTR region of the element in the wheat genome.

Supplementary Fig. 7



Supplementary Figure 7 | Uncropped gel images. (a) Uncropped gel image of supplementary figure 1a. (b) Uncropped gel images for RT-PCR in supplementary figure 1c. (c) Uncropped gel image for expression pattern analysis in figure 2a.

Supplementary Tables

Supplementary Table 1. Estimates of nucleotide diversity and test of statistics for selection in *Ms2*

	n	π (per site)	θ (per site)	Tajima's D	Fu and Li's D
Wild	30	0.00615	0.00585	0.176 ^{\$}	0.381 ^{\$}
Landrace	28	0.00386	0.00233	2.026*	0.704 ^{\$}
Modern Variety	26	0.00302	0.00238	0.847 ^{\$}	0.720 ^{\$}

Note : π : nucleotide diversity , θ : Watterson's estimator of genetic diversity, Statistical significance: \$ P>0.1, * P <0.05

Supplementary Table 2. Materials were used to explore the nucleotide diversity of *Ms2*

Accession/Sample names	Species	Abbr. Population Name
TA1618	<i>Aegilops tauschii meyeri</i>	wild diploid D
TA2456	<i>Aegilops tauschii strangulata</i>	wild diploid D
TA2457	<i>Aegilops tauschii typica</i>	wild diploid D
TA2460	<i>Aegilops tauschii typica</i>	wild diploid D
TA2464	<i>Aegilops tauschii strangulata</i>	wild diploid D
TA2561	<i>Aegilops tauschii typica</i>	wild diploid D
TA1645	<i>Aegilops tauschii strangulata</i>	wild diploid D
TA1662	<i>Aegilops tauschii var.tauschii</i>	wild diploid D
TA2461	<i>Aegilops tauschii var.typica</i>	wild diploid D
TA2463	<i>Aegilops tauschii strangulata</i>	wild diploid D
TA2474	<i>Aegilops tauschii var.tauschii</i>	wild diploid D
TA2475	<i>Aegilops tauschii var.tauschii</i>	wild diploid D
TA2529	<i>Aegilops tauschii var.meyeri</i>	wild diploid D
CLAE1	<i>Aegilops tauschii</i>	wild diploid D
CLAE5	<i>Aegilops tauschii</i>	wild diploid D
CLAE17	<i>Aegilops tauschii</i>	wild diploid D
CLAE25	<i>Aegilops tauschii</i>	wild diploid D
PI 268210	<i>Aegilops tauschii</i>	wild diploid D
RL5214	<i>Aegilops tauschii</i>	wild diploid D
RL5257	<i>Aegilops tauschii</i>	wild diploid D
RL5261	<i>Aegilops tauschii</i>	wild diploid D
RL5263	<i>Aegilops tauschii</i>	wild diploid D
RL5272	<i>Aegilops tauschii</i>	wild diploid D
RL5392	<i>Aegilops tauschii</i>	wild diploid D
RL5422	<i>Aegilops tauschii</i>	wild diploid D
Y221	<i>Aegilops tauschii ssp.Stran gulata Eig.</i>	wild diploid D
Y222	<i>Aegilops tauschii ssp.strangulata EIG.</i>	wild diploid D
Y293	<i>Aegilops tauschii ssp.meyeri GRISEB.</i>	wild diploid D
Y294	<i>Aegilops tauschii ssp.meyeri GRISEB.</i>	wild diploid D
Y296	<i>Aegilops tauschii ssp.strangulata EIG.</i>	wild diploid D
sankecun	<i>Triticum asetivum</i>	landrace
zijuhong	<i>Triticum asetivum</i>	landrace
lanxizaoxiaomai	<i>Triticum asetivum</i>	landrace
heshangmai	<i>Triticum asetivum</i>	landrace
hongmangyouzimai	<i>Triticum asetivum</i>	landrace
tumangmai	<i>Triticum asetivum</i>	landrace
jiyumai	<i>Triticum asetivum</i>	landrace
changmangyingkema	<i>Triticum asetivum</i>	landrace
nuomai	<i>Triticum asetivum</i>	landrace
wuxumai	<i>Triticum asetivum</i>	landrace
baihuamai	<i>Triticum asetivum</i>	landrace

dahongmai	<i>Triticum aestivum</i>	landrace
xinganxiaomai	<i>Triticum aestivum</i>	landrace
sanyuehuang	<i>Triticum aestivum</i>	landrace
dabaimai	<i>Triticum aestivum</i>	landrace
huangguaxian	<i>Triticum aestivum</i>	landrace
baiyoumai	<i>Triticum aestivum</i>	landrace
huajiechangbaican	<i>Triticum aestivum</i>	landrace
sanyuanmai	<i>Triticum aestivum</i>	landrace
shanmai	<i>Triticum aestivum</i>	landrace
sifangmai	<i>Triticum aestivum</i>	landrace
tuotuomai	<i>Triticum aestivum</i>	landrace
yangmai	<i>Triticum aestivum</i>	landrace
qigongmai	<i>Triticum aestivum</i>	landrace
chiyacao	<i>Triticum aestivum</i>	landrace
changgongfangxingmai	<i>Triticum aestivum</i>	landrace
yunnanxiaomai (YN3)	<i>Triticum aestivum</i>	landrace
paozimai	<i>Triticum aestivum</i>	landrace
bainong 791	<i>Triticum aestivum</i>	cultivated line
chuanmai 22	<i>Triticum aestivum</i>	cultivated line
chuanmai 42	<i>Triticum aestivum</i>	cultivated line
fengkang 7	<i>Triticum aestivum</i>	cultivated line
jinan 16	<i>Triticum aestivum</i>	cultivated line
jinan 4	<i>Triticum aestivum</i>	cultivated line
jinan 8	<i>Triticum aestivum</i>	cultivated line
jimai 23	<i>Triticum aestivum</i>	cultivated line
jingnong 79-13	<i>Triticum aestivum</i>	cultivated line
kefeng 6	<i>Triticum aestivum</i>	cultivated line
kehan 10	<i>Triticum aestivum</i>	cultivated line
liaochun 9	<i>Triticum aestivum</i>	cultivated line
longmai 33	<i>Triticum aestivum</i>	cultivated line
lumai 3	<i>Triticum aestivum</i>	cultivated line
mianyang 29	<i>Triticum aestivum</i>	cultivated line
neimai 19	<i>Triticum aestivum</i>	cultivated line
ningmai 13	<i>Triticum aestivum</i>	cultivated line
nongda 36	<i>Triticum aestivum</i>	cultivated line
shanhe 6	<i>Triticum aestivum</i>	cultivated line
shannong 1	<i>Triticum aestivum</i>	cultivated line
shijiazhuang 407	<i>Triticum aestivum</i>	cultivated line
wanmai 50	<i>Triticum aestivum</i>	cultivated line
xibei 612	<i>Triticum aestivum</i>	cultivated line
xiannong 39	<i>Triticum aestivum</i>	cultivated line
xiaoyan 4	<i>Triticum aestivum</i>	cultivated line
xiaoyan 759	<i>Triticum aestivum</i>	cultivated line

Supplementary Table 3. Primers used in map-based cloning and RT-qPCR

primer name	sequence 5' – 3'	type
M1-Forward	GGTATGCTCCTCATTTATTCC	SSR
M1-Reverse	GACTAGTTCATCTTGCATTCAC	
M2-Forward	TAGTATTTGAGAGAGAGGAGCTG	SSR
M2-Reverse	TTTTGTTCGTCCTGATCTGA	
M3-Forward	TCGGATCCTTCACTGATGTGCT	SNP
M3-Reverse	TCCTGGCAAGTCTGCATCTTCA	
M4-Forward	TGCACTTTAGAGCTCTGCACCA	SNP
M4-Reverse	GGTGAGTGTGTGTGTTTGTGAGCA	
M5-Forward	TTGGCTTGCCATTGCCATCGTC	SNP
M5-Reverse	CAAACCTTGCAGCCTCAACTTG	
M6-Forward	AGGGTGAGTAAGATCGTCAGCA	SNP
M6-Reverse	GAAGTACTGATTGATGTGAGGAC	
M7-Forward	GGCGTCCTCGTCTCCCAATA	Insertion
M7-Reverse	CGATGATGAACTCACCTGCTGTA	
M8-Forward	CCATACGAAAGCATAGCAATA	SSR
M8-Reverse	GTGATTGTGAGCAGTCCATT	
M9-Forward	CCACAAAATCAATGGTTTCTA	SSR
M9-Reverse	AACATTTTTAGACGTTTGACG	
M10-Forward	GGAGGCATAATGGAGAATG	SSR
M10-Reverse	CGTCTGTTTTCTCTCTCATCA	
M11-Forward	CTTACATGCAGATCCGTCTAAT	SSR
M11-Reverse	ATTTGTGTGTGTGTCTCTCTCT	
TaGAPDH-Forward	TTAGACTTGCGAAGCCAGCA	
TaGAPDH-Reverse	AAATGCCCTTGAGGTTTCCC	
TaTUBULIN-Forward	TGAGGACTGGTGCTTACCGC	
TaTUBULIN-Reverse	GCACCATCAAACCTCAGGGA	
Ms2-RT-Forward	CTGCTGCATCCGACTAACTATC	
Ms2-RT-Reverse	TGAGAATACTGTCCACCAAACCTC	