

Chromatin modification contributes to the expression divergence of three *TaGS2* homoeologs in hexaploid wheat

Wei Zhang ^{a,b,1}, Xiaoli Fan ^{d,1}, Yingjie Gao ^{c,1}, Lei Liu ^{a,b}, Lijing Sun ^e, Qiannan Su ^{a,f}, Jie Han ^{a,f}, Na Zhang ^{a,f}, Fa Cui ^a, Jun Ji ^{a,b}, Yiping Tong ^b, Junming Li ^{a,b,2}

^a Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang 050022, China

^b State Key Laboratory of Plant Cell and Chromosome Engineering, Chinese Academy of Sciences, Beijing 100101, China

^c Hebei Key Laboratory of Molecular and Cellular Biology, Hebei Normal University, Shijiazhuang 050024, China

^d Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

^e Institute of Cereal and Oil Crops, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang 050035, China

^f University of Chinese Academy of Sciences, Beijing 100049, China

¹ These authors contributed equally to this work.

² Address correspondence to ljm@sjziam.ac.cn.

Tel: +86-311-85887272

Fax: +86-311-85815093

Supplementary Data

| | | | | | | | | |
|--------------|--|---|--|----------------------------|---------------|-----------------------------|-------------------|-----|
| TMB02-AA.seq | ATGGCGCAGGCGGTGGTCCGGCGATGCACTGCCAGTGGGGC | CGGGGGCAGGTCGGCCGTCGCCGGCAGGCGAGCCCG | CGCAGGGTGTGGGCGTCA | 100 | | | | |
| TH02-SS.seq | ATGGCGCAGGCGGTGGTCCGGCGATGCACTGCCAGTGGGGC | CGGGGGCAGGTCGGCCGTCGCCGGCAGGCGAGCCCG | CGCAGGGTGTGGGCGTCA | 100 | | | | |
| Z415-SS.seq | ATGGCGCAGGCGGTGGTCCGGCGATGCACTGCCAGTGGGGC | CGGGGGCAGGTCGGCCGTCGCCGGCAGGCGAGCCCG | CGCAGGGTGTGGGCGTCA | 100 | | | | |
| Y199-DD.seq | ATGGCGCAGGCGGTGGTCCGGCGATGCACTGCCAGTGGGGC | CGGGGGCAGGTCGGCCGTCGCCGGCAGGCGAGCCCG | CGCAGGGTGTGGGCGTCA | 100 | | | | |
| Consensus | atggcgagggcggtgggtccggcgatgcactgccagtgggg | cgggggcaggtcggccgctccggcgagggcagcccg | ggcaggggtgtgggcgctca | | | | | |
| TMB02-AA.seq | GGAGGACCGCCCGCCGCTCTGGTTCAAGTGTCTGCTCGCC | CTGGCCCGGAGACCACCGGGTCCATCCAGAGGATGCAGCAGCTGCTCGACATGGACAC | 200 | | | | | |
| TH02-SS.seq | GGAGGACCGCCCGCCGCTCTGGTTCAAGTGTCTGCTCGCC | CTGGCCCGGAGACCACCGGGTCCATCCAGAGGATGCAGCAGCTGCTCGACATGGACAC | 200 | | | | | |
| Z415-SS.seq | GGAGGACCGCCCGCCGCTCTGGTTCAAGTGTCTGCTCGCC | CTGGCCCGGAGACCACCGGGTCCATCCAGAGGATGCAGCAGCTGCTCGACATGGACAC | 200 | | | | | |
| Y199-DD.seq | GGAGGACCGCCCGCCGCTCTGGTTCAAGTGTCTGCTCGCC | CTGGCCCGGAGACCACCGGGTCCATCCAGAGGATGCAGCAGCTGCTCGACATGGACAC | 200 | | | | | |
| Consensus | ggagaccgcccgcgcccctcgggtcaagtgctgcctcggcccgagaccacggg | gtcatccagaggatgcagcagctgctcgacatggacac | | | | | | |
| TMB02-AA.seq | CACGCCCTTACCAGCAAGATCATCGCCGAGTACATCTGGGTGGAGGATCTGGAATTGACCTCAG | AGCAAAATCAAGGACGATTTCC | AAAGCCAGTGGAG | 300 | | | | |
| TH02-SS.seq | CACGCCCTTACCAGCAAGATCATCGCCGAGTACATCTGGGTGGAGGATCTGGAATTGACCTCAG | AGCAAAATCAAGGACGATTTCC | AAAGCCAGTGGAG | 300 | | | | |
| Z415-SS.seq | CACGCCCTTACCAGCAAGATCATCGCCGAGTACATCTGGGTGGAGGATCTGGAATTGACCTCAG | AGCAAAATCAAGGACGATTTCC | AAAGCCAGTGGAG | 300 | | | | |
| Y199-DD.seq | CACGCCCTTACCAGCAAGATCATCGCCGAGTACATCTGGGTGGAGGATCTGGAATTGACCTCAG | AGCAAAATCAAGGACGATTTCC | AAAGCCAGTGGAG | 300 | | | | |
| Consensus | cacgcccttaccagcaagatcatcggcgagtagctgggtggaggtctggaattgacctcag | agcaaatcaaggacgatttc | aagccagtggag | | | | | |
| TMB02-AA.seq | GACCCCTCAGAGTACCCAAATGGAACACTACGACGGATCGAGCAGCGG | CAGGCTCCTGGAGAAGACAGTGAAGTATCCTATACCCACAGGC | ATATTCA | 400 | | | | |
| TH02-SS.seq | GACCCCTCAGAGTACCCAAATGGAACACTACGACGGATCGAGCAGCGG | CAGGCTCCTGGAGAAGACAGTGAAGTATCCTATACCCACAGGC | ATATTCA | 400 | | | | |
| Z415-SS.seq | GACCCCTCAGAGTACCCAAATGGAACACTACGACGGATCGAGCAGCGG | CAGGCTCCTGGAGAAGACAGTGAAGTATCCTATACCCACAGGC | ATATTCA | 400 | | | | |
| Y199-DD.seq | GACCCCTCAGAGTACCCAAATGGAACACTACGACGGATCGAGCAGCGG | CAGGCTCCTGGAGAAGACAGTGAAGTATCCTATACCCACAGGC | ATATTCA | 400 | | | | |
| Consensus | gaccctcagagctacc | aaatggaactacgacggatcgagcacaagg | caggctcctggagaagacagtggaagtcatcctataccacaggc | atattca | | | | |
| TMB02-AA.seq | AGGACCCATTCCGAGGAGGCAACAACATCTGGTTATCTGTGACACCTACACGCCACAAGGGGAACCCATCCCTAC | AAACAA | CGACACATGGTGCACA | 500 | | | | |
| TH02-SS.seq | AGGACCCATTCCGAGGAGGCAACAACATCTGGTTATCTGTGACACCTACACGCCACAAGGGGAACCCATCCCTAC | AAACAA | CGACACATGGTGCACA | 500 | | | | |
| Z415-SS.seq | AGGACCCATTCCGAGGAGGCAACAACATCTGGTTATCTGTGACACCTACACGCCACAAGGGGAACCCATCCCTAC | AAACAA | CGACACATGGTGCACA | 500 | | | | |
| Y199-DD.seq | AGGACCCATTCCGAGGAGGCAACAACATCTGGTTATCTGTGACACCTACACGCCACAAGGGGAACCCATCCCTAC | AAACAA | CGACACATGGTGCACA | 500 | | | | |
| Consensus | aggaccattccgagggagggcaacaacat | ctggttatctgtgacacctacacgccacaaggggaacccatccctac | aacaa | cgacacatggctgcaca | | | | |
| TMB02-AA.seq | AATCTTACGTCAGCCCAAGGTCACTCTCAAGTGCCATGGTTGGAATCGAACAGGAGTACACTCTGATCGAGAGGGATG | CAACTGGCCCTTTGGCTGG | 600 | | | | | |
| TH02-SS.seq | AATCTTACGTCAGCCCAAGGTCACTCTCAAGTGCCATGGTTGGAATCGAACAGGAGTACACTCTGATCGAGAGGGATG | CAACTGGCCCTTTGGCTGG | 600 | | | | | |
| Z415-SS.seq | AATCTTACGTCAGCCCAAGGTCACTCTCAAGTGCCATGGTTGGAATCGAACAGGAGTACACTCTGATCGAGAGGGATG | CAACTGGCCCTTTGGCTGG | 600 | | | | | |
| Y199-DD.seq | AATCTTACGTCAGCCCAAGGTCACTCTCAAGTGCCATGGTTGGAATCGAACAGGAGTACACTCTGATCGAGAGGGATG | CAACTGGCCCTTTGGCTGG | 600 | | | | | |
| Consensus | aatctcagtcagcccaaggtcactc | caagtgccatgggttggaaatcgaaacagggatgacctctgatcgagagggatg | aactggccctttggctgg | | | | | |
| TMB02-AA.seq | CTGTGGAGGGTA | CCTGGCCCCCAGGGTCCATACTACTGCGC | GTAGGATCAGACAA | TCATTGGCCG | SACAT | TCG | GATGCTCACTACAAGGC | 700 |
| TH02-SS.seq | CTGTGGAGGGTA | CCTGGCCCCCAGGGTCCATACTACTGCGC | GTAGGATCAGACAA | TCATTGGCCG | SACAT | TCG | GATGCTCACTACAAGGC | 700 |
| Z415-SS.seq | CTGTGGAGGGTA | CCTGGCCCCCAGGGTCCATACTACTGCGC | GTAGGATCAGACAA | TCATTGGCCG | SACAT | TCG | GATGCTCACTACAAGGC | 700 |
| Y199-DD.seq | CTGTGGAGGGTA | CCTGGCCCCCAGGGTCCATACTACTGCGC | GTAGGATCAGACAA | TCATTGGCCG | SACAT | TCG | GATGCTCACTACAAGGC | 700 |
| Consensus | ctgtggagggta | cctggccccagggttccataactactgctgc | gtaggatcagacaa | tcaattggccg | gacat | tc | gatgctcactacaaggc | t |
| TMB02-AA.seq | GCCTTTACGCTGGAATTGAAATCAGTGGAAACAACCGGGAGGTCATGCTGGTTCAGTGGGAGTACACAGGTTGG | CTAGCGTTGGTATTGATG | AGGAGA | 800 | | | | |
| TH02-SS.seq | GCCTTTACGCTGGAATTGAAATCAGTGGAAACAACCGGGAGGTCATGCTGGTTCAGTGGGAGTACACAGGTTGG | CTAGCGTTGGTATTGATG | AGGAGA | 800 | | | | |
| Z415-SS.seq | GCCTTTACGCTGGAATTGAAATCAGTGGAAACAACCGGGAGGTCATGCTGGTTCAGTGGGAGTACACAGGTTGG | CTAGCGTTGGTATTGATG | AGGAGA | 800 | | | | |
| Y199-DD.seq | GCCTTTACGCTGGAATTGAAATCAGTGGAAACAACCGGGAGGTCATGCTGGTTCAGTGGGAGTACACAGGTTGG | CTAGCGTTGGTATTGATG | AGGAGA | 800 | | | | |
| Consensus | gcctttacgc | ggaattgaaatcagtggaacaacacgggaggtcatgctggctcagtgaggatgaccaggttgg | cctagcgttggattatgagc | ggaga | | | | |
| TMB02-AA.seq | TCACATATGGGCTTCAAGATACATTCCTCGAGAGAATCAGGAGGAC | GCTGGAGTCTGTGCTCAC | TTTGACCC | AAACCAATCCAGGGTACTGGAATGGA | 900 | | | |
| TH02-SS.seq | TCACATATGGGCTTCAAGATACATTCCTCGAGAGAATCAGGAGGAC | GCTGGAGTCTGTGCTCAC | TTTGACCC | AAACCAATCCAGGGTACTGGAATGGA | 900 | | | |
| Z415-SS.seq | TCACATATGGGCTTCAAGATACATTCCTCGAGAGAATCAGGAGGAC | GCTGGAGTCTGTGCTCAC | TTTGACCC | AAACCAATCCAGGGTACTGGAATGGA | 900 | | | |
| Y199-DD.seq | TCACATATGGGCTTCAAGATACATTCCTCGAGAGAATCAGGAGGAC | GCTGGAGTCTGTGCTCAC | TTTGACCC | AAACCAATCCAGGGTACTGGAATGGA | 900 | | | |
| Consensus | tcacatatgggcttcaagatatactctcgagagaatcaggagacg | gctgg | gt | gtgctcac | tttgacc | aaaccaatccagggtagctggaatgga | | |
| TMB02-AA.seq | CTGTGCTGCACACAATTACAGCACCTTGAGCATGCGT | SAGGATGGAGGTTTCGACGTGATCAAGAAGGCAATCCT | CAACCTTTCAC | TCGCCATGAT | 1000 | | | |
| TH02-SS.seq | CTGTGCTGCACACAATTACAGCACCTTGAGCATGCGT | SAGGATGGAGGTTTCGACGTGATCAAGAAGGCAATCCT | CAACCTTTCAC | TCGCCATGAT | 1000 | | | |
| Z415-SS.seq | CTGTGCTGCACACAATTACAGCACCTTGAGCATGCGT | SAGGATGGAGGTTTCGACGTGATCAAGAAGGCAATCCT | CAACCTTTCAC | TCGCCATGAT | 1000 | | | |
| Y199-DD.seq | CTGTGCTGCACACAATTACAGCACCTTGAGCATGCGT | SAGGATGGAGGTTTCGACGTGATCAAGAAGGCAATCCT | CAACCTTTCAC | TCGCCATGAT | 1000 | | | |
| Consensus | gctgctgcccacacaattacagcac | ttgagcatgctg | gaggtatggaggttttcgacgtgatcaagaagggcaatcct | aacctttcact | cgccatga | t | | |
| TMB02-AA.seq | TGCACATAGCCGATATGTTGAAGGAAACGAAACGGAGG | TGACAGGCTTACATGAGACAGTAGCATATCAGACTTCTCATGGGG | CTC | CAACCGTGG | 1100 | | | |
| TH02-SS.seq | TGCACATAGCCGATATGTTGAAGGAAACGAAACGGAGG | TGACAGGCTTACATGAGACAGTAGCATATCAGACTTCTCATGGGG | CTC | CAACCGTGG | 1100 | | | |
| Z415-SS.seq | TGCACATAGCCGATATGTTGAAGGAAACGAAACGGAGG | TGACAGGCTTACATGAGACAGTAGCATATCAGACTTCTCATGGGG | CTC | CAACCGTGG | 1100 | | | |
| Y199-DD.seq | TGCACATAGCCGATATGTTGAAGGAAACGAAACGGAGG | TGACAGGCTTACATGAGACAGTAGCATATCAGACTTCTCATGGGG | CTC | CAACCGTGG | 1100 | | | |
| Consensus | tgcacatagccgatatggtgaaggaacgaacggaggtg | tgacaggcttacatgagacagtagcatatcagacttctcatgggg | ctc | caac | cgctgg | | | |
| TMB02-AA.seq | CTGCTCATTCGTTGGGGCAGAGSACTGAGGCAAGGGCAAAGGATACCTGGAGGA | CGTCGCCCGGC | TCGAACATGGACCC | TACACCGTACGGGG | 1200 | | | |
| TH02-SS.seq | CTGCTCATTCGTTGGGGCAGAGSACTGAGGCAAGGGCAAAGGATACCTGGAGGA | CGTCGCCCGGC | TCGAACATGGACCC | TACACCGTACGGGG | 1200 | | | |
| Z415-SS.seq | CTGCTCATTCGTTGGGGCAGAGSACTGAGGCAAGGGCAAAGGATACCTGGAGGA | CGTCGCCCGGC | TCGAACATGGACCC | TACACCGTACGGGG | 1200 | | | |
| Y199-DD.seq | CTGCTCATTCGTTGGGGCAGAGSACTGAGGCAAGGGCAAAGGATACCTGGAGGA | CGTCGCCCGGC | TCGAACATGGACCC | TACACCGTACGGGG | 1200 | | | |
| Consensus | ctgctcattcgttggggcagag | sactgaggcaagggcaaaaggatacctggagga | cgtcgcccggc | tccaacatggacc | tacacctgacggg | | | |
| TMB02-AA.seq | CTGCTGGCCGAGACCAGATCCTGTGGGAGCCGACCTC | SAGGCGGAGGCCCTCGCTGCCAAGAAGTGGCGCTGAAGGTATGA | 1284 | | | | | |
| TH02-SS.seq | CTGCTGGCCGAGACCAGATCCTGTGGGAGCCGACCTC | SAGGCGGAGGCCCTCGCTGCCAAGAAGTGGCGCTGAAGGTATGA | 1284 | | | | | |
| Z415-SS.seq | CTGCTGGCCGAGACCAGATCCTGTGGGAGCCGACCTC | SAGGCGGAGGCCCTCGCTGCCAAGAAGTGGCGCTGAAGGTATGA | 1284 | | | | | |
| Y199-DD.seq | CTGCTGGCCGAGACCAGATCCTGTGGGAGCCGACCTC | SAGGCGGAGGCCCTCGCTGCCAAGAAGTGGCGCTGAAGGTATGA | 1284 | | | | | |
| Consensus | ctgctggc | gagaccagatcctgtgggagccgacct | saggcgaggccctcgctgccaaagagctggcgctgaaggtatga | | | | | |

Fig S1. cDNA sequences alignment of TaGS2 homoeologs in the progenitor species .

| | | |
|------------------|---|------|
| TaGS2-A-cDNA.seq | ATGGCCAGGCGGTGGTCCCGCGATGCAGTCCAGGTGGCGTCCGGGCGAGTGGCCGTCCTCCCGCGAGGCAGCCCGGGGAGGGTGTGGGCGTCA | 100 |
| TaGS2-B-cDNA.seq | ATGGCCAGGCGGTGGTCCCGCGATGCAGTCCAGGTGGCGTCCGGGCGAGTGGCCGTCCTCCCGCGAGGCAGCCCGGGGAGGGTGTGGGCGTCA | 100 |
| TaGS2-D-cDNA.seq | ATGGCCAGGCGGTGGTCCCGCGATGCAGTCCAGGTGGCGTCCGGGCGAGTGGCCGTCCTCCCGCGAGGCAGCCCGGGGAGGGTGTGGGCGTCA | 100 |
| Consensus | atggccagggcgggtggtgcccgcgatgcagtgccaggtggcgtccgggaggtggccgtcctcccgcgaggcagcccggggaggggtgtgggcgctca | |
| TaGS2-A-cDNA.seq | GGAGGACCCCGCGCCACTCCCGCTTCAAGGTGCTGGCCCTCGGCCCGGAGACCACCGGCGTATCCAGAGGATGCAGCAGCTGCTGCAGATGGACAC | 200 |
| TaGS2-B-cDNA.seq | GGAGGACCCCGCGCCACTCCCGCTTCAAGGTGCTGGCCCTCGGCCCGGAGACCACCGGCGTATCCAGAGGATGCAGCAGCTGCTGCAGATGGACAC | 200 |
| TaGS2-D-cDNA.seq | GGAGGACCCCGCGCCACTCCCGCTTCAAGGTGCTGGCCCTCGGCCCGGAGACCACCGGCGTATCCAGAGGATGCAGCAGCTGCTGCAGATGGACAC | 200 |
| Consensus | ggaggaccgcccgcgcaactccggttcaaggtgctggccctcgcccggagaccacggcgctcatccagaggatgcagcagctgctgcagatggacac | |
| TaGS2-A-cDNA.seq | CACGCCCTCACCAGCAAGATCATCGCCGAGTACATCTGGTGGAGGATCTGGAATTGACCTCAGAGCAAAATCAAGGACGATTTGAAGCCAGTGGAG | 300 |
| TaGS2-B-cDNA.seq | CACGCCCTCACCAGCAAGATCATCGCCGAGTACATCTGGTGGAGGATCTGGAATTGACCTCAGAGCAAAATCAAGGACGATTTGAAGCCAGTGGAG | 300 |
| TaGS2-D-cDNA.seq | CACGCCCTCACCAGCAAGATCATCGCCGAGTACATCTGGTGGAGGATCTGGAATTGACCTCAGAGCAAAATCAAGGACGATTTGAAGCCAGTGGAG | 300 |
| Consensus | cagcccttcaccgacaagatcatcgccgagtagatctgggtggaggatctggaattgacctcagaagcaaatcaaggacgatttcgaagccagtgag | |
| TaGS2-A-cDNA.seq | GACCCCTCAGAGCTACCAAAATGAAATTTAGACGGATCGAGCACGGCCAGGCTCTGGAGAAGACAGTGAAGTCATCTATACCCACAGGCCATATTTCA | 400 |
| TaGS2-B-cDNA.seq | GACCCCTCAGAGCTACCAAAATGAAATTTAGACGGATCGAGCACGGCCAGGCTCTGGAGAAGACAGTGAAGTCATCTATACCCACAGGCCATATTTCA | 400 |
| TaGS2-D-cDNA.seq | GACCCCTCAGAGCTACCAAAATGAAATTTAGACGGATCGAGCACGGCCAGGCTCTGGAGAAGACAGTGAAGTCATCTATACCCACAGGCCATATTTCA | 400 |
| Consensus | gacccctcagagctaccgaaatggaactagacggatcgagcacaggcgaggctcctggagaagacagtgaaagtcaictctataccacagggccatattca | |
| TaGS2-A-cDNA.seq | AGGACCCATCCGAGGAGGCACAACATCTGGTTATCTGTGACACCTACAGCCACAAAGGGGAACCCATCCCTACTAAACAACGACACATGGCTGCACA | 500 |
| TaGS2-B-cDNA.seq | AGGACCCATCCGAGGAGGCACAACATCTGGTTATCTGTGACACCTACAGCCACAAAGGGGAACCCATCCCTACTAAACAACGACACATGGCTGCACA | 500 |
| TaGS2-D-cDNA.seq | AGGACCCATCCGAGGAGGCACAACATCTGGTTATCTGTGACACCTACAGCCACAAAGGGGAACCCATCCCTACTAAACAACGACACATGGCTGCACA | 500 |
| Consensus | aggaccatccgaggaggcaacaacatctggttatctgtgacacctacagccacaaggggaacccatccctactaacaagcgacacatggctgcaca | |
| TaGS2-A-cDNA.seq | AATCTTCAGTGAACCAAGGTCACTGCACAAGTCCATGGTTTGGAAATCGAACAGGAGTACACTCTGATGCAGAGGGATGTGAACCTGGCTCTTTGGCTGG | 600 |
| TaGS2-B-cDNA.seq | AATCTTCAGTGAACCAAGGTCACTGCACAAGTCCATGGTTTGGAAATCGAACAGGAGTACACTCTGATGCAGAGGGATGTGAACCTGGCTCTTTGGCTGG | 600 |
| TaGS2-D-cDNA.seq | AATCTTCAGTGAACCAAGGTCACTGCACAAGTCCATGGTTTGGAAATCGAACAGGAGTACACTCTGATGCAGAGGGATGTGAACCTGGCTCTTTGGCTGG | 600 |
| Consensus | aatcttcagtgacccaaggtcactgcacaagtgccatggtttggaatcgaaacaggagtagactctgatgcagagggatgtagaactggcctcttggctgg | |
| TaGS2-A-cDNA.seq | CCTGTTGGAGGTTACCTGGCCCGAGGGTCCATCTACTCGCCCGTAGGATCAGACAAGTCATTGGCCGTGACATATCGATGCTCACTACAAGGCT | 700 |
| TaGS2-B-cDNA.seq | CCTGTTGGAGGTTACCTGGCCCGAGGGTCCATCTACTCGCCCGTAGGATCAGACAAGTCATTGGCCGTGACATATCGATGCTCACTACAAGGCT | 700 |
| TaGS2-D-cDNA.seq | CCTGTTGGAGGTTACCTGGCCCGAGGGTCCATCTACTCGCCCGTAGGATCAGACAAGTCATTGGCCGTGACATATCGATGCTCACTACAAGGCT | 700 |
| Consensus | cctgttggagggtaccctggcccaggggtccatctactgcccgtaggatcagacaagtcatltagccgtgacatccgatgctcaactacaaggct | |
| TaGS2-A-cDNA.seq | GCCTTACCGCGGAATGAAATCAGTGAACAACGGGAGGTTCATGCTGTCAGTGGAGTACCAGGTTGGACCTTAGGCTTGGTATTGATGCCGGAGA | 800 |
| TaGS2-B-cDNA.seq | GCCTTACCGCGGAATGAAATCAGTGAACAACGGGAGGTTCATGCTGTCAGTGGAGTACCAGGTTGGACCTTAGGCTTGGTATTGATGCCGGAGA | 800 |
| TaGS2-D-cDNA.seq | GCCTTACCGCGGAATGAAATCAGTGAACAACGGGAGGTTCATGCTGTCAGTGGAGTACCAGGTTGGACCTTAGGCTTGGTATTGATGCCGGAGA | 800 |
| Consensus | gccttacggggaatgaaatcagtgaaacaacgggaggtcatgctggtcagtgaggtagaccaggttggacctagcgttggatitgatgcccggaga | |
| TaGS2-A-cDNA.seq | CCACATATGGCTTCAAGATACATCTCGAGAGAATCAGGAGCAAGCTGGTGTGCTGCTACCCTTGACCAAAAACCAATCCAGGGTGACTGGAATGGA | 900 |
| TaGS2-B-cDNA.seq | CCACATATGGCTTCAAGATACATCTCGAGAGAATCAGGAGCAAGCTGGTGTGCTGCTACCCTTGACCAAAAACCAATCCAGGGTGACTGGAATGGA | 900 |
| TaGS2-D-cDNA.seq | CCACATATGGCTTCAAGATACATCTCGAGAGAATCAGGAGCAAGCTGGTGTGCTGCTACCCTTGACCAAAAACCAATCCAGGGTGACTGGAATGGA | 900 |
| Consensus | ccacatattggcttcaagatcatctcgagagaatcaggagcaagctggtgtggtgctcacccttgaccaaaaaccaatccagggtagctggaatgga | |
| TaGS2-A-cDNA.seq | GCTGGCTGCCACAAAATACAGTACATGAGCATCCCGAATGAGGAGTTTGCAGCTGATCAAGAAGGCAATCTGAACCTTTCACTTCGGCATGACT | 1000 |
| TaGS2-B-cDNA.seq | GCTGGCTGCCACAAAATACAGTACATGAGCATCCCGAATGAGGAGTTTGCAGCTGATCAAGAAGGCAATCTGAACCTTTCACTTCGGCATGACT | 1000 |
| TaGS2-D-cDNA.seq | GCTGGCTGCCACAAAATACAGTACATGAGCATCCCGAATGAGGAGTTTGCAGCTGATCAAGAAGGCAATCTGAACCTTTCACTTCGGCATGACT | 1000 |
| Consensus | gctggctgccacaaaattacagtagcatcccgaaatgagagtttgcagctgatcaagaaggcaatctgaacctttcacttcggcatgact | |
| TaGS2-A-cDNA.seq | TGCACATAGCCGCATATGTTGAAGGAAACGACGGAGCTGACAGGGCTACAGGAGACAGTACATTTAGACTTCTCATGGGCTGTCCGAACTGTGG | 1100 |
| TaGS2-B-cDNA.seq | TGCACATAGCCGCATATGTTGAAGGAAACGACGGAGCTGACAGGGCTACAGGAGACAGTACATTTAGACTTCTCATGGGCTGTCCGAACTGTGG | 1100 |
| TaGS2-D-cDNA.seq | TGCACATAGCCGCATATGTTGAAGGAAACGACGGAGCTGACAGGGCTACAGGAGACAGTACATTTAGACTTCTCATGGGCTGTCCGAACTGTGG | 1100 |
| Consensus | tgcacatagccgatattggtgaaggaaacgacggaggttgacagggctacaggagacagtagcatttagacttctcatgggctgtccgaaactgtgg | |
| TaGS2-A-cDNA.seq | CTGCTCTATTCTGTGGCCGAGACCGGAGGCAAAAGGCAAAAGGATACCTGGAGGACCGTCCGCCGGCTCGAACATGGACCCATACACCGTGACGGCG | 1200 |
| TaGS2-B-cDNA.seq | CTGCTCTATTCTGTGGCCGAGACCGGAGGCAAAAGGCAAAAGGATACCTGGAGGACCGTCCGCCGGCTCGAACATGGACCCATACACCGTGACGGCG | 1200 |
| TaGS2-D-cDNA.seq | CTGCTCTATTCTGTGGCCGAGACCGGAGGCAAAAGGCAAAAGGATACCTGGAGGACCGTCCGCCGGCTCGAACATGGACCCATACACCGTGACGGCG | 1200 |
| Consensus | ctgctctattctgtggccgagaccggagggcaaaaggcaaaaggatacctggaggacctcgcccggcgtcgaacatggaccctacacctgacggcg | |
| TaGS2-A-cDNA.seq | CTGCTGGCCGAGACACGATCCTGTGGAGCCGACCTCGAGGCCGAGGCCCTCGTCCCAAGAAGCTGGCGCTGAAGGTATGA | 1284 |
| TaGS2-B-cDNA.seq | CTGCTGGCCGAGACACGATCCTGTGGAGCCGACCTCGAGGCCGAGGCCCTCGTCCCAAGAAGCTGGCGCTGAAGGTATGA | 1284 |
| TaGS2-D-cDNA.seq | CTGCTGGCCGAGACACGATCCTGTGGAGCCGACCTCGAGGCCGAGGCCCTCGTCCCAAGAAGCTGGCGCTGAAGGTATGA | 1284 |
| Consensus | ctgctggccgagaccagatcctgtggagcccgacctcgaggccgaggccctcgctgccaaagactggcgctgaaggatgga | |

Fig S3. Sequence alignment of the cDNA sequences of *TaGS2-A*, *TaGS2-B* and *TaGS2-D* in hexaploid wheat

Kn9204.

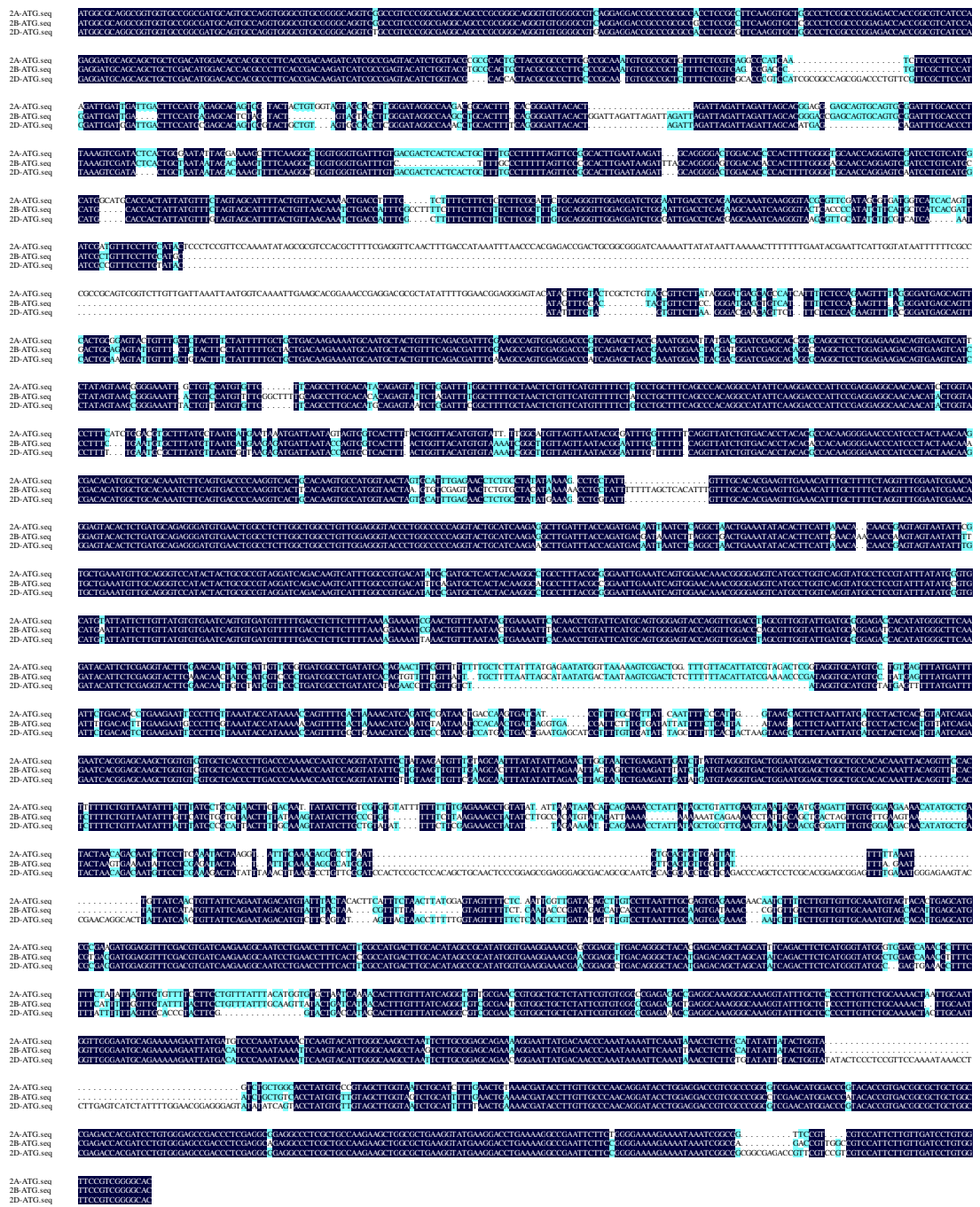


Fig S5. Sequence alignment of the DNA sequences of TaGS2-A, TaGS2-B and TaGS2-D in hexaploid wheat Kn9204.

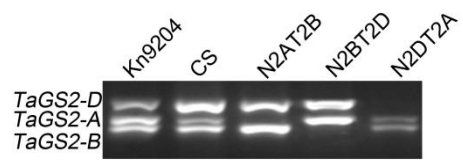


Fig S6. Chromosome mapping of the three *TaGS2* homoeologous genes in Chinese Spring (CS) nulli-tetrasomic lines. The line names specify which chromosomes are present (e.g., N2AT2B is nulli-2A tetra-2B). Kn9204, wild-type plant. The genomic origin of the PCR products is indicated on the left.

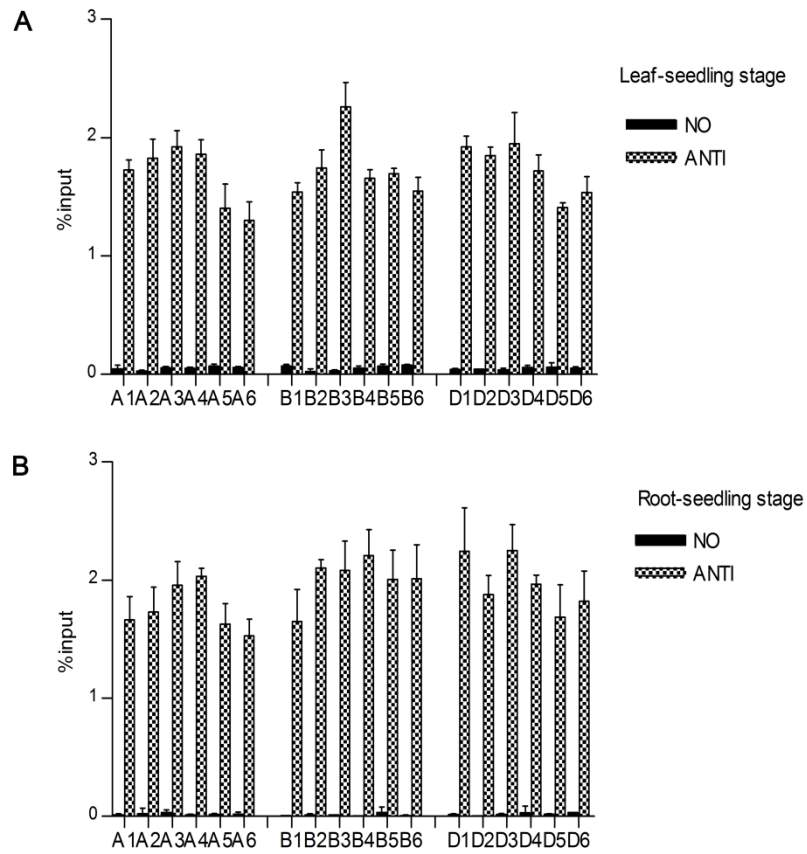


Fig S7. ChIP with anti-H3K9me2 antibodies to determine the levels of H3K9me3 at *TaGS2* in WT.

(A) Leaf of 10-d-old seedling.

(B) Root of 10-d-old seedling.

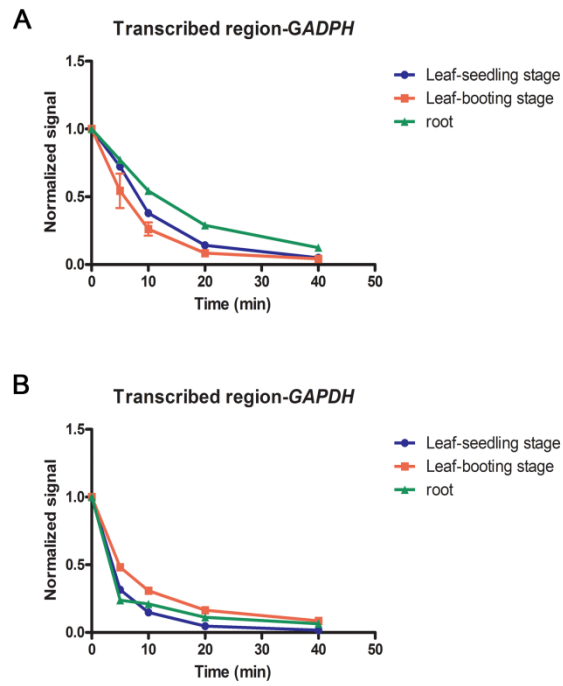


Fig S8. Accessibility of transcribed region of *GAPDH*.
(A) Accessibility of transcribed regions to Micrococcal Nuclease.
(B) Accessibility of transcribed regions to Dnase I.

Table S1. Primers used for PCR, RT-PCR and real-time RT-PCR analysis.

| Experiment | Name | Primer sequence (5'-3') |
|--------------------------------|-------------|---------------------------------|
| genomic PCR | | |
| | WLF-B | TCCCTCCCTCCTCTCCTC |
| | WLF-D | TCCCTCCGTCCCCTCCTC |
| | WLF1-A | GCGGAGTAAGTAAGTAAGCAG |
| | F1spAR1-A | TCCCTATAAGAACGCTACAGAG |
| | F1spAF1-A | TGTCATGGCATGGCATGCAC |
| | GS2R6 | ATGTTGTTGCCTCCTCGGAATG |
| | GS2F5 | ACCCACAGGCCATATTCAAGG |
| | GS2R4 | AGCCAGCTCCATTCCAGTCA |
| | GS2F2 | TCGAGAGAATCACGGAGCAA |
| | WLR1 | GGTCCTTCATACCTTCAGCG |
| promoter genomic PCR | | |
| | GS2-A016 F1 | ACAAACGGAATCTGACTTTGAAAAGTTTGCC |
| | GS2-A016 R1 | CGCCGCTGCTTACTTACTTACTC |
| | GS2-B017 F1 | CAAGGTCTGGTAATCCCATGTGTTGTGC |
| | GS2-B017 R1 | CGCCGCTGCTTGCTTACTTACTTACTCC |
| | GS2-D018 F2 | CCTGCTATTGCTTCGGATGA |
| | GS2-D018 R1 | CGCCGCTACTTACTTACTTACTC |
| RT-PCR | | |
| | mGS2-F2 | TCCTCCCTCGTCTCGTCCGCGT |
| | mGS2-R2 | AGTGCCCCGACGGAACCACAGG |
| <i>TaGS2</i> mutants selection | | |
| | In10F | TGGCTGCCACACAAATTACAG |
| | In10R | CCTTCTTGATCACGTGCGAAAC |
| ChIP | | |
| | qPCR-A-F1 | TTGGTTTCTGGACCGGTTTG |
| | qPCR-A-R1 | CAGTGGCATCTGGCTCTTGA |
| | qPCR-A-F2 | GCGTGCTTGTCCGTCTCTCT |
| | qPCR-A-R2 | ATCAGCGAATGAACGCAACA |
| | qPCR-A-F3 | TGCAGGGTTGGAGGATCTG |
| | qPCR-A-R3 | CTATCGAACGCGTACCCTTGA |
| | qPCR-A-F4 | GCCCACAGGCCATATTCAAG |
| | qPCR-A-R4 | AGATGAAAGGTACCAGGATGTTGTT |
| | qPCR-A-F5 | TTTGCGAGTGAGAAACAACAATC |
| | qPCR-A-R5 | ATCTTCGCGCATGCTCAGT |
| | qPCR-A-F6 | ACGCCCTTCACCGACAAG |
| | qPCR-A-R6 | GCGCCTGACGAGAAAACAG |

| | |
|-----------|--------------------------|
| qPCR-B-F1 | CGTCCCCGTCGTTGTCTCT |
| qPCR-B-R1 | ACGAACAAGCATTGCATCGA |
| qPCR-B-F2 | GGCTGGGCTGGGTATGG |
| qPCR-B-R2 | GGCAAAAAACCGTCCAAAGA |
| qPCR-B-F3 | TCAGAAGCAAATCAAGGGTACTCA |
| qPCR-B-R3 | GCAAACCTATGCATGCAAGGAAA |
| qPCR-B-F4 | CCCGATAGAGCATCACCTTAATTT |
| qPCR-B-R4 | ACGCATGCTCAATGTGCTACAT |
| qPCR-B-F5 | CCCTTCACCGACAAGATCATC |
| qPCR-B-R5 | GGGTCCGCTGACGAGAAAA |
| qPCR-B-F6 | CATTTGGCCGTGACATTTCA |
| qPCR-B-R6 | TGATTTCAATTCCGGCGTAAAG |
| qPCR-D-F1 | CGTCCGCGTCCTTGTCA |
| qPCR-D-R1 | GAGAATCGAGGAAGCATTGCA |
| qPCR-D-F2 | GGCAGCCGCATCTTTCAT |
| qPCR-D-R2 | GCCCAGAATGCACACACATC |
| qPCR-D-F3 | ATCATCGCCGAGTACATCTGGTA |
| qPCR-D-R3 | GACGCGTGCGACGAGAA |
| qPCR-D-F4 | TCATCAAATATCGCCGTTTCC |
| qPCR-D-R4 | AGTGAAGTCTCATCCCGTAAAA |
| qPCR-D-F5 | CTGACCGAATGAGCATCCTTTT |
| qPCR-D-R5 | ACACCAGCTTGCTCCGTGAT |
| qPCR-D-F6 | GAACCTTTCACTTCGCCATGA |
| qPCR-D-R6 | TTCACTCGCCATACCCATGA |

BSP

| | |
|----------|------------------------------------|
| BSP-A-F1 | TAGGTTATTAGTGTGGTTGTATTTGTATAAGAAG |
| BSP-A-R1 | TAACCCCRAAACCAATAATTA |
| BSP-A-F2 | ATGTTGTATTTGATAAAGGGGT |
| BSP-A-R2 | AATATTCCTTCCCTCTAACTCC |
| BSP-A-F3 | GTGTGTGTTTTTTTTGGGTAGA |
| BSP-A-R3 | TAATCAACCAACCAAAAACAAC |
| BSP-A-F4 | TGTTTTGGTTTGGTTGATTAGT |
| BSP-A-R4 | ATCAATCAATCTATAAAAACRAAAAT |
| BSP-B-F1 | TGTATTGTAGTYGGGTTATTGTAG |
| BSP-B-R1 | TCATCCCCTTTATCAAATACA |
| BSP-B-F2 | GTATTTGATAAAGGGGATGAAGG |
| BSP-B-R2 | CCAAAAAAAACACACACATCTC |
| BSP-B-F3 | GAGATGTGTGTGTTTTTTTTGG |
| BSP-B-R3 | AACCAAAAACAAAAAAAACAAC |
| BSP-B-F4 | GTTTTTTTGTGTTTTGGTTGATTAG |
| BSP-B-R4 | CAATCAATCCATAAAAACRAAC |
| BSP-D-F1 | GTTTATGTGTGTTGTTGTTGGG |
| BSP-D-R1 | CAAACCAACCAAAATACAACA |

| | |
|----------|----------------------------|
| BSP-D-F2 | TGTTGTATTTGGTTGGGTT |
| BSP-D-R2 | AATCRCRCTAAAAATATTTATC |
| BSP-D-F3 | ATATTTGATAAAGGGGATGAAGG |
| BSP-D-R3 | CAATATATTCCTTATTCCTTCTCTCC |
| BSP-D-F4 | GGAGAGAAGGAATAAGGAATATATTG |
| BSP-D-R4 | CAAAACAAAAAAAACAAAACCA |
| BSP-D-F5 | TTTTTTGTTGAAGGTTTGGAG |
| BSP-D-R5 | TAAAAATCAATCCATCAATCCA |

TaqMan absolute quantification real-time RT-PCR

| | |
|----------|-----------------------------------|
| tubb2-F | ATGGTTCTTGACAACGAGGCT |
| tubb2-R | AGGCGAGGGAATGGGATAA |
| tubb2-P | ATCTGTTTCAGGACTCTTAAGCTGACCACAC |
| TaGS2A-F | ACAAGATCATCGCCGAGTACATCTG |
| TaGS2A-R | ACTGTCTTCTCCAGGAGCCTGCCCCG |
| TaGS2A-P | CCCGTCAGAGCTACCCAAATGGAATTATGAC |
| TaGS2B-F | ACAAGATCATCGCCGAGTACATCTG |
| TaGS2B-R | ACTGTCTTCTCCAGGAGCCTGGCCT |
| TaGS2B-P | CCCGTCAGAGCTACCGAAATGGAAGTACGAT |
| TaGS2D-F | AGTACATCTGGGTTGGAGGATCTGG |
| TaGS2D-R | TGTGCTCGATCCGTCGTAGTT |
| TaGS2D-P | CCTCAGGAGCAAATCAAGGACGATTTCAAAGCC |

SYBR relative quantification real-time RT-PCR

| | |
|---------|----------------------|
| qGS2-F1 | CCGAGACCACGATCCTGT |
| qGS2-R1 | GGTCCTTCATACCTTCAGCG |

Enzyme accessibility assay

| | |
|----------|----------------------|
| qGAPDH-F | TTAGACTTGCGAAGCCAGCA |
| qGAPDH-R | AAATGCCCTTGAGGTTTCCC |

*R: A or G; Y: C or T