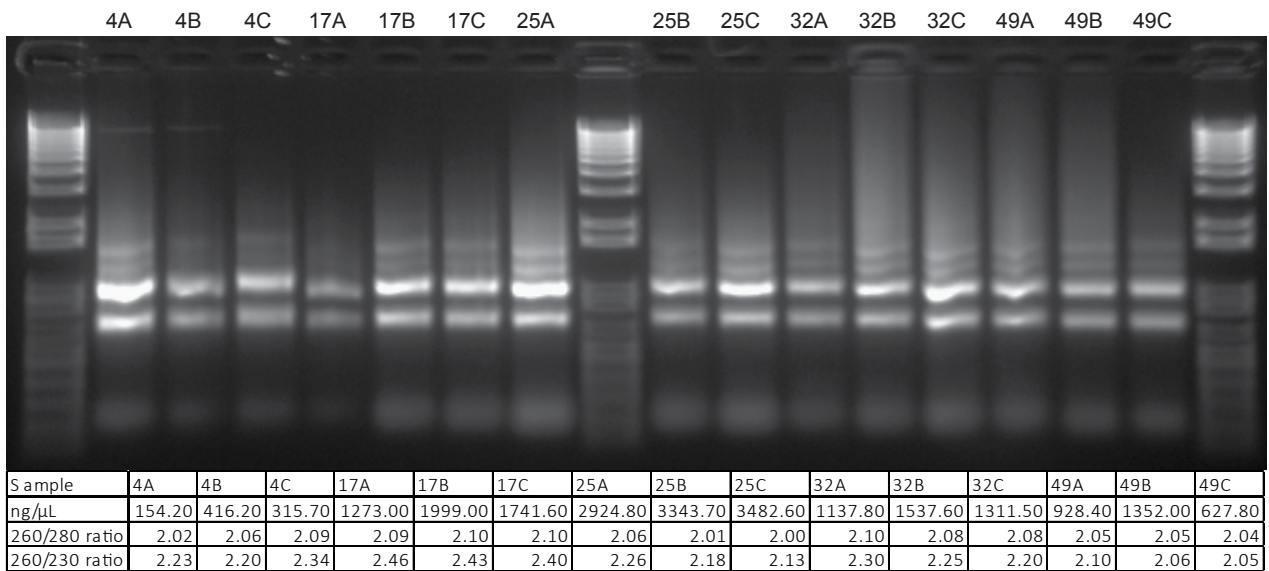
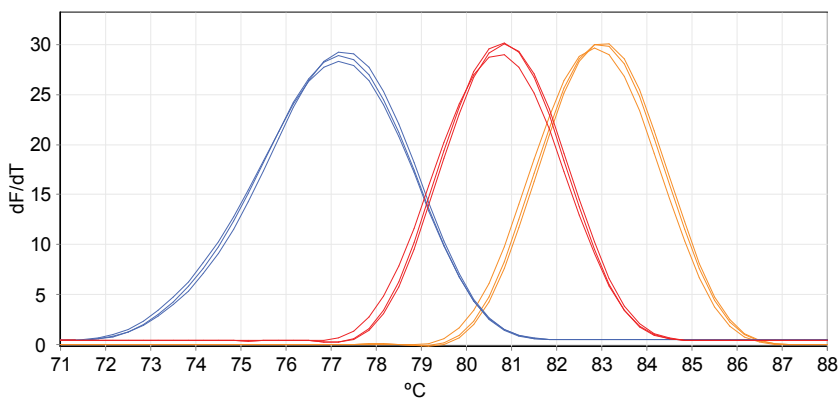


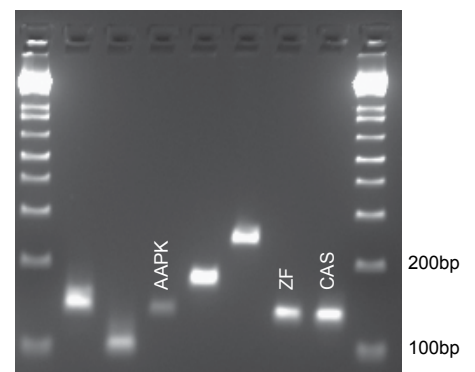
Additional file 1A. Expression profile of CAS measured by qPCR in the developing grains at day 4, 17, 25, 32 and 49 days after anthesis.



Additional file 1B. The integrity, concentration and purity of the RNA used for the qPCR analysis, analyzed on a 1% agarose gel and with Nanodrop spectrophotometer (table below gel).



Additional file 1C. Melting curve analysis for the three genes used for the qPCR analysis. AAPK (blue), ZF (red), CAS (orange).



Additional file 1D. qPCR product run on 4% agarose gel and visualized with GelRed™. The size of the products corresponds to the DNA sequence they are supposed to replicate: AAPK (140bp), ZF (132bp) and CAS (133bp).

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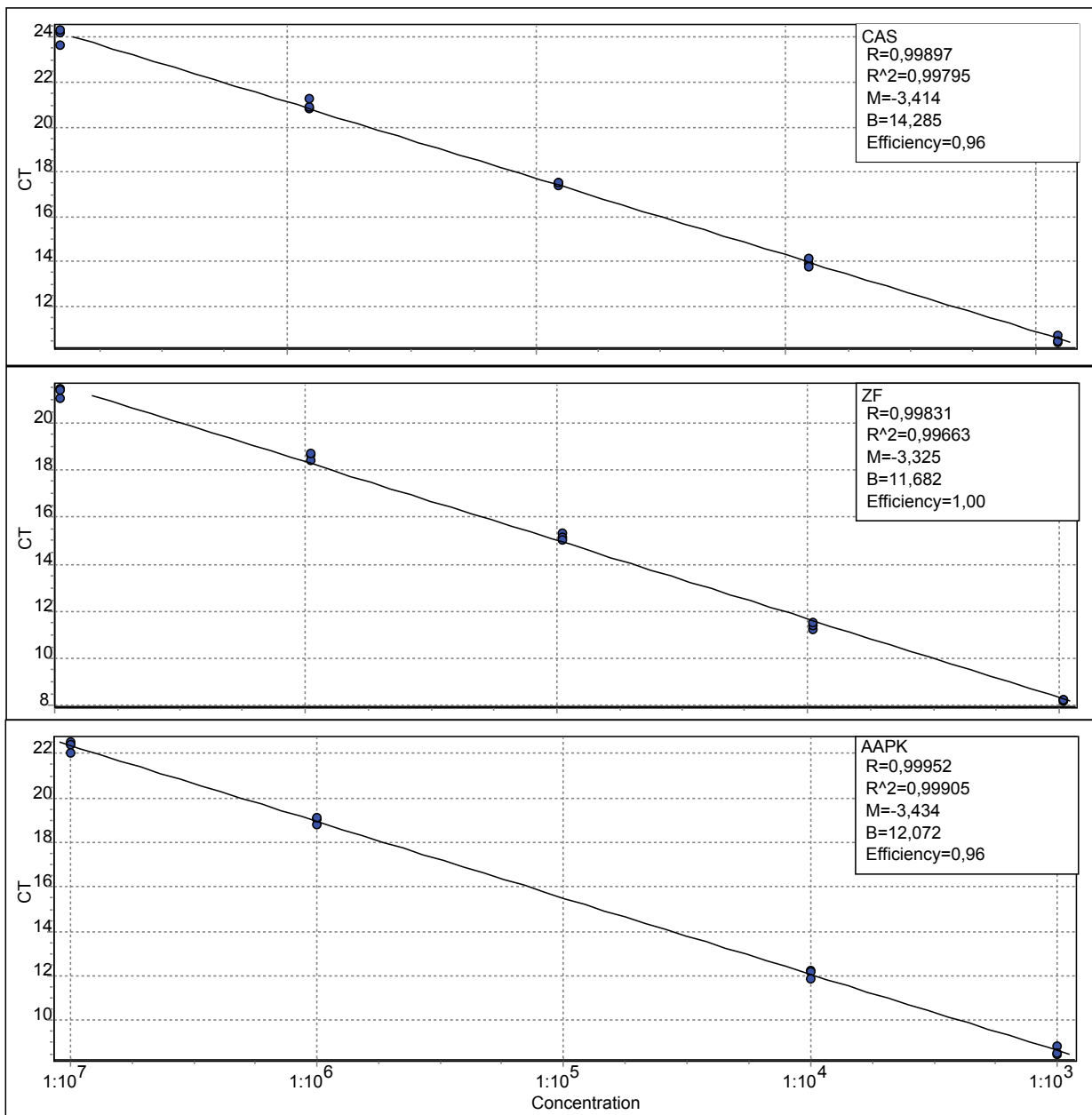
CAS
Consensus      CATTGTGACTGTTCTTCCAAGCCTTGGGGAGCGATACTTGTTCATCTGCGCTGTTTGTAGAGTTGAGAAAGGAGGCTGAAGCAATGGAACCACTGCCGGTG
Sequencing     ~~~~~~TGTCCTCTGCGCTGTTTGTAGAGTTGAGAAAGGAGGCTGAAGCAATGGAACCACTGCCGGTG
                GATTAATTTGGCCAATCAAACCGGGCACTGGAA
                |||
                GATTAATTTGGCCAATCAAACCGGGCACTAGGA

ZF
Consensus      TCCACCAGCACTCAGGTTTCATTGAGCGCTGTAGTTTGTCTCTAACTGATATGGTGTTCATTGAGAAGGACTATTTGGAACATGAGTTAAACTGCATCT
Sequencing     ~~~~~~TTGTCGTA~CTGATATGGTGTTCATTGAGAAGGACTATTTGGAACATGAGTTAAACAGCATCT
                CTG~GTCCTGGGATTCAAAGTACCGAGTCCAGCT
                |||
                CAGAGTCCCAGGATTCAAAGTACCGAGTCCAGCT

AAPK
Consensus      GGAATGGTGCATTATGCCGTGTTAATCCAGCAAATCTG~TATTAGATTAATTAAGAAGGCTTCTGTTATGTTTATACTATGCCATTTCTTTGGAAGTT
Sequencing     ~~~~~~CAATTCTGATGTTAGATTAATTAAGAAGGCTTCTGTTATGTTTATACTATGCCATTTCTTTGGAAGTT
                TGGATAGTTTGTCTGATCAAATGCGTGGTAACCATTAGGAG
                |||
                TGGATAGTTTGTCTGATCAAATGCGTGGTAACCATTAGGAG

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Additional file 1E. Alignment of Sanger sequencing result against the specific genes of interest CAS, ZF and AAPK. The primer binding sites for the forward and reverse primers are marked in red font.



Additional file 1F. Efficiency test of the primers specific for the genes CAS, ZF and AAPK on serial dilutions of template in the following dilutions: 1:10³, 1:10⁴, 1:10⁵, 1:10⁶, 1:10⁷.