CORRIGENDUM

Corrigendum: Enzymatic study on AtCCD4 and AtCCD7 and their potential to form acyclic regulatory metabolites

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The original published version of this article contained inaccurate information within the first paragraph of the Materials and Methods section of the article. The paragraph should read as follows:

pThio-AtCCD4: The intron-free AtCCD4 (At4g19170) gene was amplified from genomic DNA using the primers: A3-forward: 5’-AGGAGAGCAATGGACTCTGTT-3’ and A3-reverse: RP 5’-TTAAAGCTTATTAAGGTCACT-3’, which cover the whole coding sequence (start ATG and bases complementary to the stop codon are underlined). The resulting PCR product was purified using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ), and cloned into pCR2.1®-TOPO® vector (Invitrogen, Paisley, UK), according to the instructions of the manufacturer and yielding pA3-TOPO. The AtCCD4 fragment, including coding sequence and 9 bp upstream of the start ATG (s. primer A3-forward), was then isolated from pA3-TOPO, using EcoRI, and ligated into accordingly digested and dephosphorylated pThio-Dan1 (Trautmann et al., 2013), a plasmid made from the commercially available pBAD/THIO-TOPO®TA (Invitrogen, Paisley, UK) by inserting the multiple cloning site of pUC18. Sequencing of the resulting expression vector pThio-Dan1-AtCCD4 unraveled a point mutation downstream of the sole SacI restriction site of AtCCD4 (base 584–589 in the coding sequence). To correct this mutation, we amplified the AtCCD4 3′-region (starting with base 581 in the coding sequence) from genomic DNA using the primers SacI-FP 5’-CCGGAGCTCCGTTATGCTACGTG-3’ and A3-reverse: RP 5’-TTAAAGCTTATTAAGGTCACT-3’, which cover the whole coding sequence (start ATG and bases complementary to the stop codon are underlined). The resulting PCR product was purified using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ), and cloned into pCR2.1®-TOPO® vector (Invitrogen, Paisley, UK), according to the instructions of the manufacturer and yielding pA3-TOPO. The AtCCD4 fragment, including coding sequence and 9 bp upstream of the start ATG (s. primer A3-forward), was then isolated from pA3-TOPO, using EcoRI, and ligated into accordingly digested and dephosphorylated pThio-Dan1 (Trautmann et al., 2013), a plasmid made from the commercially available pBAD/THIO-TOPO®TA (Invitrogen, Paisley, UK) by inserting the multiple cloning site of pUC18. Sequencing of the resulting expression vector pThio-Dan1-AtCCD4 unraveled a point mutation downstream of the sole SacI restriction site of AtCCD4 (base 584–589 in the coding sequence). To correct this mutation, we amplified the AtCCD4 3′-region (starting with base 581 in the coding sequence) from genomic DNA using the primers SacI-FP 5’-CCGGAGCTCCGTTATGCTACGTG-3’ and A3-reverse: RP 5’-TTAAAGCTTATTAAGGTCACT-3’, which cover the whole coding sequence (start ATG and bases complementary to the stop codon are underlined). The resulting PCR product was purified using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ), and cloned into pCR2.1®-TOPO® vector (Invitrogen, Paisley, UK), according to the instructions of the manufacturer and yielding pA3-TOPO. The AtCCD4 fragment, including coding sequence and 9 bp upstream of the start ATG (s. primer A3-forward), was then isolated from pA3-TOPO, using EcoRI, and ligated into accordingly digested and dephosphorylated pThio-Dan1-AtCCD4, replacing the corresponding mutation-containing fragment and leading to pThio-AtCCD4. The integrity of pThio-AtCCD4 was confirmed by sequencing. The plasmid contains the whole AtCCD4 coding sequence flanked by 9 and 8 non-coding bases upstream of the start codon and following the stop codon, respectively.


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