

1. Supplementary Materials

1.1. The stabilization of the epigenetic changes in the ODN6- treated flax after multiple passages in the *in vitro* conditions

Due to the low level of transfer to the next generation of epigenetic changes induced by ODN6, the stability of the obtained changes was monitored in the *in vitro* culture during passages.

In the *in vitro* cultured ODN6- treated flax the level of the CHS gene expression was assessed (Fig. S1A). Similarly to the primarily observed changes, after multiple passages in the ODN6 - treated flax the repression of the CHS was maintained (RQ = 0.7, in the comparison to the control set as 1).

The total methylation of the *in vitro* cultured treated with ODN6 flax was determined. Similarly to the observed originally changes, the total methylation of cytosines was elevated in the comparison to the control (Fig. S1B).

The expression of genes involved in the epigenetic modifications was investigated in the *in vitro* cultured ODN6 treated plants (Fig. S1C). The results have shown that the “epi-genes” are repressed after *in vitro* cultivation, as it was observed after 24h since the moment of incubation with oligonucleotides. Only the gene expression of CMT3 was not repressed, at the control level (set as 1).

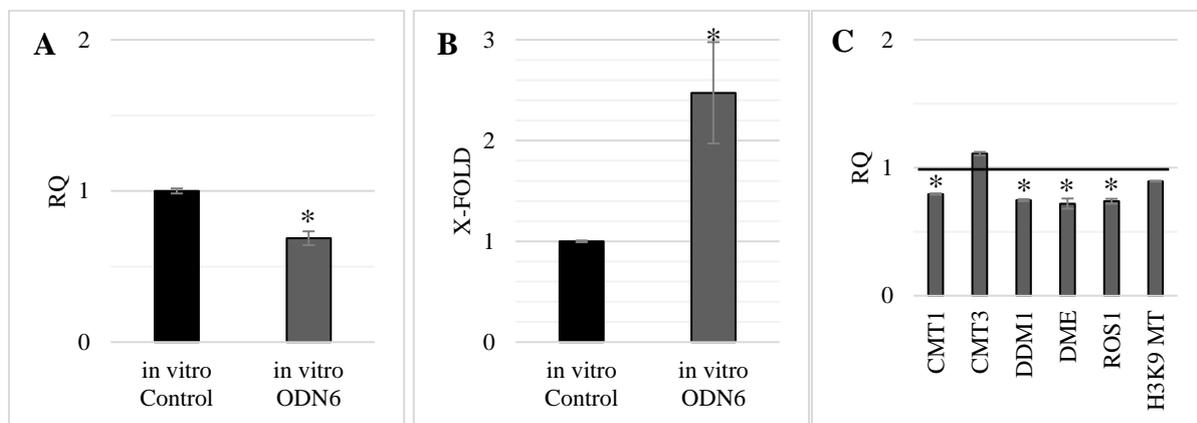


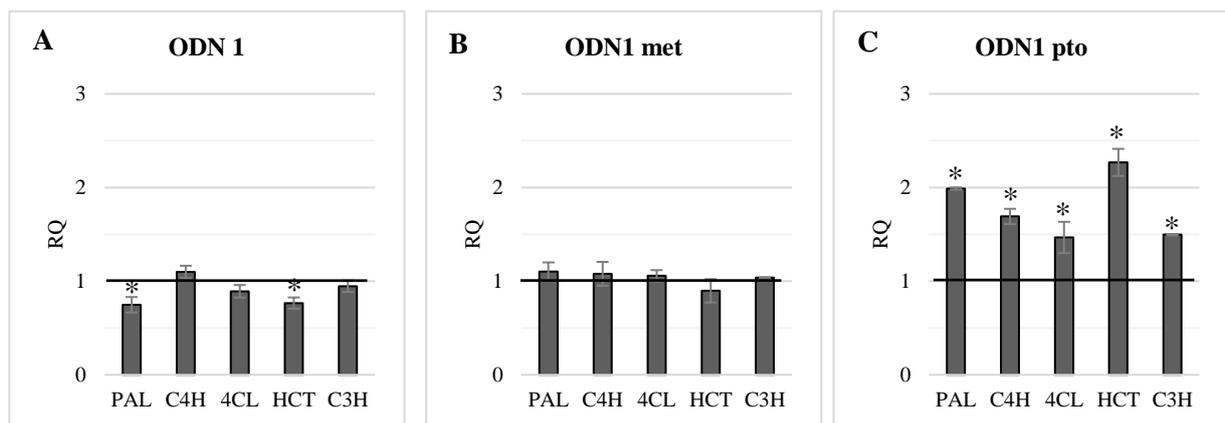
Figure S1. CHS gene expression (A), total genomic methylation (B) and expression of genes involved in epigenetic modifications (C) in the *in vitro* cultivated ODN6 treated plants. Plants after incubation with ODN were cultivated in the *in vitro* conditions and passaged every 1 to 2 months into new medium during 3 years. (A) The expression of two CHS genes, *LuCHS6* and *LuCHS7* was determined by the Real-Time PCR reaction. The values are referred to the reference gene expression actin. The Relative Quantity (RQ) presents the transcript level in the comparison to the control (set as 1, black). (B) The determination of total 5-methylcytosine in the analyzed plants was performed using a technical kit dedicated for total methylation assay (detailed description in Material and Methods section). The values are referred as x-fold in the comparison to the control (set as 1, black). (C) The expression of genes involved in epigenetic modifications was determined by the Real-Time PCR reaction. The transcript level of genes encoding following enzymes: methylases (CMT1 – chromomethylase 1, CMT3 – chromomethylase 3), demethylases (DME – DEMETER, ROS1 – repressor of silencing 1) and enzymes involved in chromatin methylation (DDM1 – decrease in DNA methylation 1, H3K9 MT – histone H3K9 methyltransferase) was presented. The values are referred to the reference gene expression actin. The Relative Quantity (RQ) presents the transcript level in the comparison to the control (set as 1, black). All presented data constitute the mean value \pm SD from at least three independent experiments. The significance of the differences between each mean and control was determined by Student’s t-test. Asterisk indicates $p < 0,05$

40 1.2. The transcript level of genes encoding other than CHS enzymes involved in the phenylpropanoid biosynthesis
41 pathway
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43 In the Figure S2 genes coding enzymes involved in the synthesis of phenylpropanoid
44 compounds (other than CHS): phenylalanine ammonia (PAL), cinnamate 4-hydroxylase (C4H), 4-
45 hydroxycinnamoyl ligase: CoA (4CL), p-hydroxycinnomoCoA transferase: shikimic/quinonic acid
46 (HCT), β -cumarate-3-hydroxylase (C3H). The transcript level of these genes were determined for plants
47 treated with: ODN1 (Fig. S2A), ODN1 met (Fig. S2B) and ODN1 pto (Fig. S2C), after 10 days from the
48 time of treatment.

49 For plants incubated with ODN1, no significant changes in gene expression for 4CL, C4H and
50 C3H was observed, in comparison to control. However, lower transcript levels were presented by genes
51 encoding PAL and HCT, for both genes RQ = 0.8 (Fig. S2A). Any significant differences in the expression
52 of phenylpropanoid metabolism genes was noted in plants incubated with ODN1 met (Fig. S2B).
53 However concerning ODN1 pto, plants showed a significant increase in expression of analyzed genes.
54 The most significant overexpression of the tested genes was reported for HCT (2.3-fold increase), PAL
55 (2-fold increase) and C4H (1.7-fold increase). Other genes encoding 4CL and C3H have shown a 1.5-fold
56 increase of the transcript level in the comparison to the control (Fig. S2C).

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59 **Figure S2.** Transcript quantity derived from genes (other than CHS) involved in the synthesis
60 phenylpropanoid compounds, determined in the plants treated with ODN1: unmodified (A),
61 methylated (B) and tiophosphorylated (C). The expression level of genes encoding following enzymes
62 was studied: PAL (phenylalanine ammonia-lyase), C4H (cinnamate-4-hydroxylase), 4CL (4-coumarate-
63 CoA ligase), HCT (hydroxycinnamoyl transferase) and C3H (cinnamate-3-hydroxylase). The values are
64 referred to the reference gene expression actin. The Relative Quantity (RQ) presents the transcript level
65 in the comparison to the control (set as 1, black). All presented data constitute the mean value \pm SD from
66 at least three independent experiments. The significance of the differences between each mean and
67 control was determined by Student's t-test. Asterisk indicates $p < 0,05$
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Target gene or sequence	Primer forward	Primer reverse	
Reference gene			
actin	5' CCGGTGTTATGGTTGGAAT 3'	5' TGTAGAAAGTGTGATGCCAAA 3'	
CHS gene expression			
<i>CHS</i> (<i>LuCHS6</i> and <i>LuCHS7</i>)	5' CCCACGTAATATTCTGCACAAGTA 3'	5' GCGCCTCGATTGTTCTC 3'	
<i>LuCHS6</i>	5' ACCATTGGGGGATAATATTGTTGG 3'	5' CTCCCGGGCTCAAGCCTC 3'	
<i>LuCHS7</i>	5' GCCATTGGGGGA TATTGTTGG 3'	5' CTCCCGGGCTGGAGCCTT 3'	
-CCGG- motif (<i>LuCHS6/LuCHS7</i>)			
coding	+552/+996	5' ACCAACTCACCATGCTCTTG 3'	5' GCGAAGTCGTCGTCAGTAGG 3'
	+775/+1219	5' CGTAGTCTGGTGCCAGATCA 3'	5' CTCGAACAACGGCCTCTCT 3'
	+829/+1273	5' GGGGCCAGACTATTGTACC 3'	5' CAATCACAATCGCCAACAAT 3'
The expression of genes encoding enzymes involved in the epigenetic modifications			
<i>CMT1</i>	5' CAGATTTTCGCTCCACAGTA 3'	5' AGAAATGTCCCATTGCTCTAT 3'	
<i>CMT3</i>	5' AAAGGGTGCTAACTTCAGG 3'	5' GACCAAATGGTTAGACGATGT 3'	
<i>DME</i>	5' ATGGCTACGGAGGCTACTTA 3'	5' TGTTTCACCTGGTGTCCATA 3'	
<i>ROS1</i>	5' GCACTGAGAAGAAGTGCC 3'	5' CTTAATGCGTGCTGCAAG 3'	
<i>DDM1</i>	5' GTGGTTAATATCTTTGTGGCAG 3'	5' CATCCAATTTGCCAGAGTAG 3'	
<i>H3K9</i>	5' TGCCAAAGGTGTAAAGCTC 3'	5' ATCCTTGCTTCGATTAGCC 3'	

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70 **Table S1.** Primers used in the Real-Time PCR reaction
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74 ATGTCGACCATCACCGTTGATGAAGTACGCAAGGCTCAACGTGCACAAGGTCCCGCCACCGT
75 CCTTGCCATCGGAACCGCCACTCCTGCCAAGTGCATCAAAGCACCTACCCTGATTACTA
76 CTTCCGTATTACCAACAGCGAGCACAAGACTGAGCTCAAGGAGAAGTTCAAGCGCATGTGTA
77 TGTTCAATTCGTATTAATTGTTATTTTATTCTAGAAATTTTCAATTTTTTGTGTCATTATTATTCATCT
78 ACGTTACGTATGTACAATCTGGAGATTGAAAAGCCCATGTTTGAATTTGTGTCCACAGCCAG
79 ACCATCCTGCCCAGATAGTGAAGGTGCTATCGACGGACACCTTCGGGAAGTGGGGTTGACTTTC
80 CACTTCTGAAAGATGTCCCCGGGCTGATTTCAAGAACATTGAGAAGAGCTTGGTGGAGGC
81 GTTTAAGCCGTTGGGGATATCGGACTGGAACCTCGCTTTTCTGGATAGCTCATCCGGGTGGTCC
82 GGCGATTCTGGACCAAGTGGAGGCTAAGTTGAACCTCAAGGAGGAGAACTGCGAGCCACG
83 AGGCAGGTTCTGGCTGATTATGGTAACATGTGAGTGTGTTGTTTCAATTTGGATGAGA
84 TGAGGAAGAAATCTGTTGCGGATGGGTTGAACACTACTGGTGAAGGGCTTATTGGGGGTTCC
85 TGTTTGGATTCCGGCCTGGACTCACTGTGGAGACTGTAGTTCTTCACAGTGTGGCTGTTTGA

86 **Figure S3.** Recognized gene fragment sequence of *LuCHS11* (Acc. no AFSQ01012744) analyzed in this
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