Materials and methods

Nuclear-extract from untreated ('U') and GA_3 treated ('T') tobacco leaves at early-vegetative growth stage (13 d after GA_3 application) was prepared using *CelLytic*^{TM}*PN* plant nuclei isolation/extraction kit (*Sigma-Aldrich*, St. Louis, USA) following the manufacturer's protocol. Proteins (~50 μg) were electrophoresed on 15% (m/v) acrylamide gels and then transferred onto *Hybond-P* (*Amersham Biosciences*, Uppsala, Sweden) polyvinylidene difluoride (PVDF) membranes. After blocking the membrane in freshly prepared Tris-buffered saline + 0.1% (v/v) *Tween-20* (TBS-T) containing 5% (m/v) non-fat dry milk, the membranes were incubated overnight with polyclonal rabbit anti-histone H4 antibody (*Abcam*, Cambridge, MA, USA) at a dilution of 1:1 000 at 4 °C with agitation, followed by incubation with an affinity purified donkey anti-rabbit horse radish peroxidase **(**HRP) conjugated IgG antibody (*GE Healthcare*, *Life Sciences*, Pittsburgh, PA, USA) at a dilution of 1:5 000 for 1 h with agitation at room temperature. Immunoreactivity was detected and exposed to X-ray *Hyperfilm*^{TM} *ECL* (*GE Healthcare*, *Life Sciences*) using an enhanced chemiluminescence, *ECL* detection reagent system (*Thermo Fisher Scientific*, San Jose, USA) and visualized on *Molecular Imager* chemiluminescence detection system (*ChemiDoc XRS*+ system, *BioRad*, Hercules, USA). The equal loading of proteins was verified by *Ponceau S* staining of membrane.

Table 1 Suppl. List of primers used in this study (F-forward, R-reverse).

Primer	Sequence (5'-3')	Accession No.	Target gene	T_m [°C]	Number of bases	Amplicon size [bp]
IME1079F	CGCGCTACACTGATGTATTC	AJ236016	18S rDNA	52	20	170
IME1079R	GTACAAAGGGCAGGGACGTA			54	20	
IMN1078F	AAGCCCATGGTTGTTGAGAC	XM_009784954	*EF-1 α*	51.8	20	105
IMN1078R	GTCAACGTTCTTGATAACAC			47.7	20	
MET1-F	TGAACCAGAAGACAAGCCGTAGT	AB280788.1	*NtMET1*	59	23	73
MET1-R	ATCTCATCCTCATTGATCTTGATGTAA			58	27	
DRM1-F	AGACCGCTTGGAGCAACTGATA	AB087883.1	*NtDRM1*	60	22	66
DRM1-R	CACGGGCTTCCACCAATTA			58	19	
CMT3-F	CCAGAGACTGCGGTAAGAAATGA	AB032538.1	*NtCMT3*	59	23	65
CMT3-R	TGCCTCCACTCCCTCAAAAG			59	20	
EXPA2-F	CCTAAATGGTGTAGAAAAG	AF049351.1	*NtEXPA2*	45	19	171
EXPA2-R	AATGCCACCTCTGTAAAT			43	18	

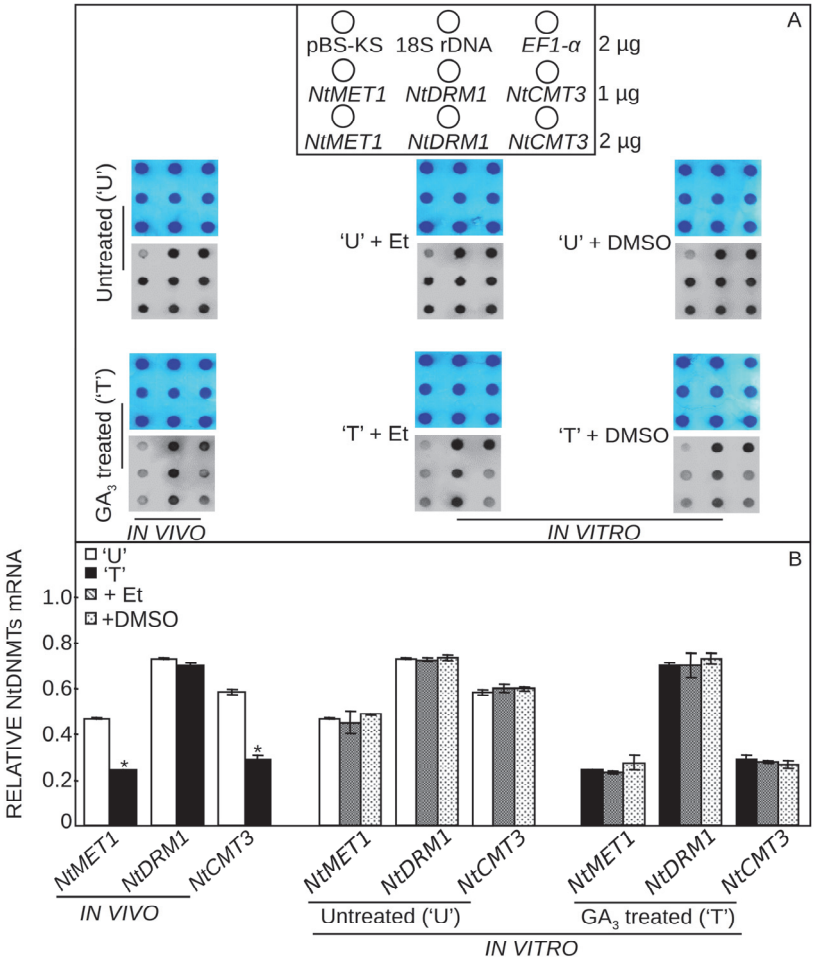

Fig.1 Suppl.Effect of solvents ethanol (Et) and dimethyl sulfoxide (DMSO) on transcriptional rate of *NtDNMTs* genes. *A* - Approximately 1 and 2 µg each of empty vector pBS-KS(+) DNA(negative control), 18S rDNA, *EF1-α* (positive controls) and *NtDNMT*s genes (*NtMET1*, *NtDRM1*,and *NtCMT3* were immobilized on charged nylon membranes (*Hybond*^{TM}-*N*$^{+}$) using a dot-blot assembly. NRO analysis of aforementioned transcripts after *in vitro* addition ofethanol (GA_3 solvent) and DMSO (cordycepin, COR solvent) to a final concentration of <0.01% (v/v) in permeabilized nuclear fractions was measured. The blots were probed with α-[^{32}P] UTP-labelled nascent NRO transcripts derived from untreated ('U') and GA_3 treated ('T') tobacco leaves. Relative position of individual dot-blot, loading control (methylene-blue stain) and recognition of dotted DNA by nuclear RNAs probes are shown in the *upper*-, *middle*- and *bottom*-panel, respectively. Hybridization signal intensity of nuclear RNA were quantified using densitometry scanning of phosphoimages. Ratio under each blot indicates the normalised nuclear RNA intensity between indicated samples. *B* - Relative signal intensity of *NtMET1*, *NtDRM1*, and *NtCMT3* transcripts (corresponding to 2 µg of dotted DNA) with respect to corresponding 18S rRNA was plotted as a *bar graph* with *asterisks* depicting the significant difference (P<0.05) as compared to corresponding "U' control. Data is representative of two independent experiments.

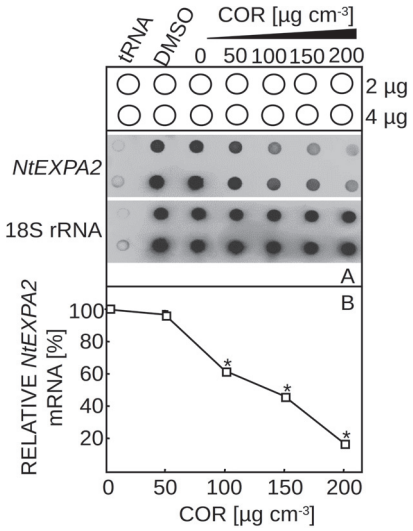

Fig. 2 Suppl. Effect of cordycepin (COR) concentration on *NtEXPA2* mRNA accumulation. COR chase assay was performed as described in Materials and methods. Ten two-week-old tobacco seedlings were pre-incubated for 30 min in incubation buffer, followed by addition of varying concentrations (0, 50, 100, 150, and 200 µg cm^{-3}) of CORto inhibit the ongoing *in vivo* transcription. As a negative control, DMSO (COR solvent) to a final conc. of <0.01% (v/v) was also supplemented in incubation buffer. Total RNAs was isolated after 2 h of chase and subjected to dot-blot hybridization with specificα-[^{32}P] ATP-labelled DNA probe of *NtEXPA2* (nucleotides: 276-444 from the start codon) (2-4 µg dot^{-1}); tRNA was used as negative control for non-specific binding of DNA probes to a random RNA fragment. Probing with 18S rDNA [nucleotides: 1467-1638 from the transcription start point **(**TSP)] was used as a loading control. *A* - A representative dot-blot (harbouring 2 and 4 µg of blotted RNA) depicting the relative position of individual dot-blot and recognition of dotted RNA by specific α-[^{32}P] dATP-labelled *NtEXPA2* and 18S rDNA probes are shown in the *upper*, *middle*, and *lower* panel,respectively. COR conc. [µg cm^{-3}] used was indicated above the schematic representation of dot-blot. *B* - The hybridization signals intensity of each nuclear RNA was quantified using densitometry scanning in a phosphoimager. Relative signal intensity [%] of *NtEXPA2* transcript (corresponding to 4 µg of dotted RNA) at each COR conc. with respect to corresponding 18S rRNA was normalized to that of 'U' (without COR addition) and plotted as a line-graph with *asterisk s*depicting the significant difference (P<0.05) as compared to 'U' control. Data is representative of two independent experiments.

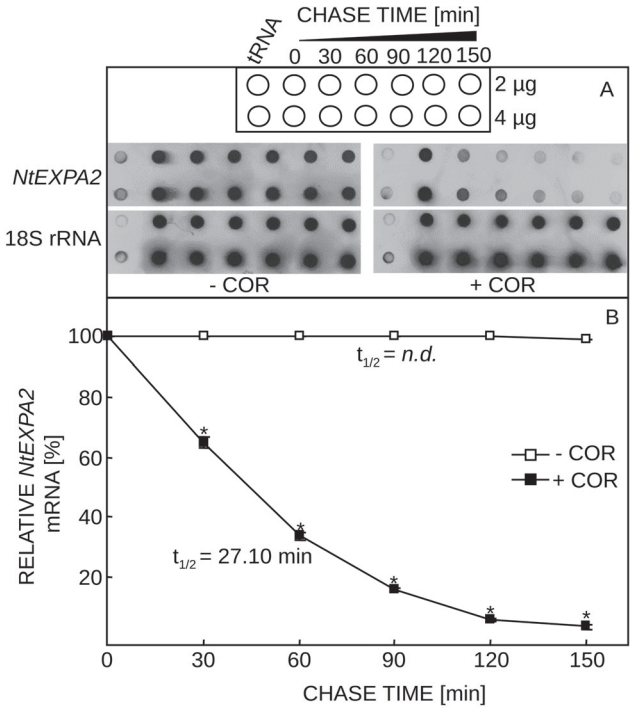

Fig.3 Suppl. Cordycepin (COR), an effective transcriptional inhibitor in *Nicotiana tabacum.* To validate whether COR blocks the nascent transcription of very unstable transcript encoded by *NtEXPA2* (AF049351.1; expansin-like protein 2, a tobacco homologue of that in *Arabidopsis thaliana*) under normal growth conditions, its mRNA decay rate in the absence or presence of optimized concentration (200 µg cm^{-3}) of COR was measured. The stable transcript encoded by 18S rDNA (AJ236016) was also used as an internal control to normalize the mRNA levels. *A* - A representative dot-blot (harbouring 2 and 4 µg of blotted RNA) depicting the relative position of individual dot-blot and recognition of dotted RNA by specific α-[^{32}P] dATP-labelled *NtEXPA2* and 18S rDNA probes are shown in the *upper*, *middle,* and *lower* panel, respectively. Time-points are indicated above the schematic representation of dot-blot. *B* - Relative signal intensity [%] of *NtEXPA2* transcript (corresponding to 4 µg of dotted RNA) at each time-point with respect to corresponding 18S rRNA was normalized to that of corresponding time T_0 and plotted as a line-graph with *asterisks* depicting the significant difference (P<0.05) as compared to corresponding 'U' (without COR). *NtEXPA2* mRNA half-life ($t_{1/2}$) was calculated by fitting non-linear regression (described in Materials and methods). Data is representative of two independent experiments. n.d.- not determined.

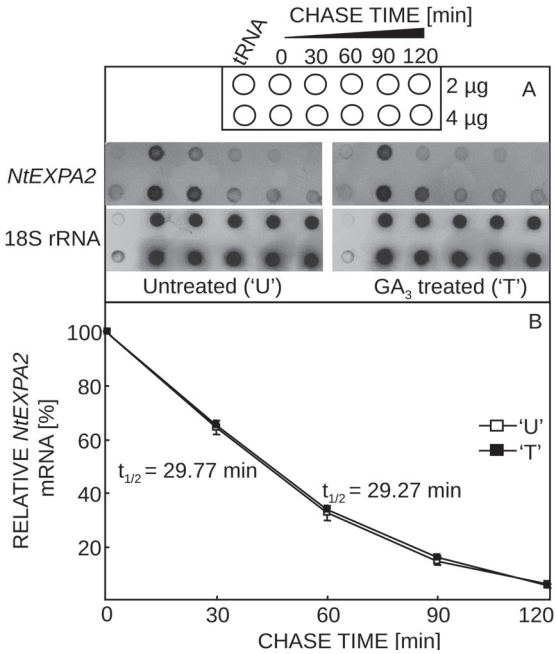

Fig.4 Suppl. *NtEXPA2* mRNA decay assay. To test whether COR blocks the nascent transcription unbiasedly in 'U' and 'T', ten two-week-old tobacco seedlings grown in the absence or presence of 50 mg dm^{-3} GA_3, were pre-incubated for 30 min in incubation buffer, followed by addition of 200 µg cm^{-3} COR to inhibit the ongoing *in vivo* transcription. *A* - A representative dot-blot (harbouring 2 and 4 µg of blotted RNA) depicting the relative position of individual dot-blot and recognition of dotted RNA by specific α-[^{32}P] dATP-labelled *NtEXPA2* and 18S rDNA probes are shown in the *upper* and *lower* panel,respectively. Time-points are indicated above the schematic representation of dot-blot. *B* - Relative signal intensity [%] of *NtEXPA2* transcript (corresponding to 4 µg of dotted RNA) at each time-point with respect to corresponding 18S rRNA was normalized to that of corresponding time T_0 and plotted as a *line-graph*. *NtEXPA2* mRNA half-life ($t_{1/2}$) was calculated by fitting non-linear regression (described in Materials and methods). Data is representative of two independent experiments.

Fig. 5 Suppl. Immunoblotting of nuclear-extracts using an anti-histone H4 antibody. The quality of the nuclear-extract was assessed by Western blot analysis using anti-histone antibody directed against the histone H4. Approximately 50 µg of nuclear extracts from untreated ('U') and GA_3-treated ('T') tobacco leaves were separated by 15% (m/v) sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and probed with anti-histone H4 antibody. The equal loading of proteins was verified by *Ponceau S* staining of membrane.

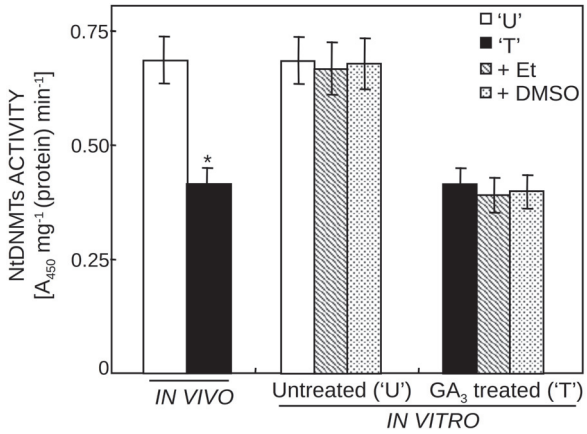

Fig. 6 Suppl. Effect of solvents ethanol (Et) and dimethyl sulfoxide (DMSO) on NtDNMTs activity. Differential NtDNMTs activity of nuclear-extract from untreated ('U') and GA_3-treated ('T') tobacco leaves, along with *in vitro* addition of ethanol (GA_3 solvent) and dimethyl sulfoxide (DMSO, GEN solvent) to a final conc. of <0.01% (v/v) was measured. Means ± SDs, *n=3*, *asterisks* indicate significant differences (P<0.05) as compared to corresponding 'U' control.